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**13. ABSTRACT (Maximum 200 words)**

This study is part of a larger multi-year study that has two main specific aims: a. the characterization of a "closed head" (lateral fluid percussion, FPI) vs a penetrating ballistic rat model of traumatic brain injury (PBI) and b. the identification of specific plasma proteomic biomarkers of TBI. Funding has covered year 1 of this study, and the following related objectives: 1. Application of TBI, neurobehavioral testing and collection of biofluids and tissues; 2. Optimization of proteomic protocols for the identification of differentially expressed proteins in the plasma of brain-traumatized rats; 3. Preliminary identification of proteins differentially expressed in plasma from rat models of TBI. All the objectives set for this part of the larger study have been met. One hundred thirty nine Sprague-Dawley rats have been used to achieve Objective 1. Results show a significant neurobehavioral effect of both kinds of TBI, more drastic for the PBI model. Optimization of proteomic technology (Objective 2) has allowed the analysis of replicate samples collected at the different time points following injury. We have thus met Objective 3 by identifying several potential time-dependent plasma protein biomarkers of TBI, including many proteins known to be normally expressed in the central nervous system. Future evaluations will need to include the verification of some of the most statistically and biologically significant plasma proteome changes observed in this study and evaluation of their dependence on trauma intensity.

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Enclosure 1

# FINAL PROGRESS REPORT

**Project:** Rat Models and Identification of Candidate Early Serum Biomarkers of Battlefield Traumatic Brain Injury

**Principal Investigator:** Fabio Leonessa, M.D.

**Contract /Grant number:** W911NF-05-1-038

**ARO proposal number:** 49021-LS-DRP

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## ORIGINAL STATEMENT OF WORK AND OBJECTIVES FOR YEAR 1

The two major goals of this study are: 1. the characterization of a penetrating ballistic *vs* a closed head model (lateral fluid percussion) of traumatic brain injury (TBI); 2. the identification of biomarkers of traumatic brain injury

Operationally, these goals translate into the following Specific Aims, as described in the original proposal for this study:

*Aim 1*.Characterization and validation of animal models of traumatic brain injury;

*Aim 2.* Optimization of proteomic protocols for the identification of differentially expressed proteins in serum* and brain tissues of TBI rat models;

*Aim 3.* Identification of proteins differentially expressed in the serum* from rat models of traumatic brain injury;

*Aim 4.* Optimization of antibody-based assays for the detection of the proteins preliminarily identified by MDLC/MS-MS approach;

*Aim 5.* Validation and characterization of proteins differentially expressed in the serum* of brain traumatized rats as candidate serum biomarkers of traumatic brain injury.

Achievement of all aims was projected over three years. As part of the overall Statement of Work, the following were the *Objectives* for year 1 of this study (which was the only part covered by the grant in question) :

    *Objective 1*:  *A*pplication of trauma (two traumatic brain injury models), functional

evaluations, collection of serum* and brain specimens. Objective 1 is aimed to support specific Aims 1 (years 1 though 3), 3 (year 1) and 5 (year 3);

*Objective 2*:  Optimization of proteomic protocols for the identification of differentially expressed proteins in the serum* of brain-traumatized rats. This objective addresses Specific Aim 2 (year 1).

*Objective 3*:  Preliminary identification of proteins differentially expressed in serum* from rat models of traumatic brain injury. Objective 3 coincides with Specific Aim 3 of this study (year 1).


*Note:  This has subsequently been changed to plasma (see below)



**EXPERIMENTAL DESIGN (year 1 objectives)**


*Objective 1: Application of traumatic brain injury, neurobehavioral testing and collection of biofluids and tissues.*


This objective is fundamental to the achievement of the two main goals of this study. The objective involves the application of the *penetrating ballistic injury* (*PBI*) and the *lateral fluid percussion injury* (*FPI*) models of TBI, at different intensities, on male Sprague-Dawley rats. Following injury (or non-injury equivalent for control groups), rats undergo neurological evaluations, except for those cases where animals are euthanized before full recovery from anesthesia is achieved.  Biosamples (primarily plasma and brains, and secondarily blood mononuclear cells and erythrocytes) are collected at the time of euthanasia, at different time points following injury time (or equivalent, in non-brain injured animals).

The plan for the full study (years 1 to 3), as outlined in the sections "Research Design" and "Timelines for Completion of Work" of the original application calls for the application of the two TBI models at three different levels of intensity over three years. All animals are to be sacrificed and biosamples collected and stored frozen at different times following injury (up to 3 days).  As described in the "Timelines for Completion of Work" section, the original plan calls for only part of this objective to be achieved during the course of year 1 (covered by this grant).

***Objective 2***      ***Optimization of proteomic protocols for the identification of differentially expressed proteins in the serum of brain-traumatized rats.***

We have made two modifications to the original experimental design: 1. we changed our target for biomarker discovery from serum to plasma; 2. we adopted a higher throughput approach to the initial proteomic identification of potential biomarkers.

At the time the initial statement of work was written we were considering using serum as our target. Technically, serum and plasma are generally considered equivalent in terms of ease of handling for liquid chromatography/mass spectrometry analyses, though a few studies may have shown the relative advantage of one with respect to the other. However, different than serum, separation of plasma allows for the collection and preservation of blood cells, another potential source for proteomic, as well as transcriptomic biomarkers.  For this reason, we have since changed our target to plasma and decided to preserve the mononuclear and erythrocyte fraction from the rat blood samples for possible separate future studies.

Initially, we foresaw the use of metabolic labeling and/or isotope coded affinity tagging (ICAT) for the initial identification and quantitation of TBI-related proteomic changes (objective 3 for year 1). This methodology allows for a more accurate quantitation of relative changes in protein concentrations already at the time of the initial liquid chromatography/mass spectrometry analyses. The disadvantage of these approaches is that they are highly time-consuming and extremely low throughput. Consequently, in the original design, we planned to carry the initial identification of TBI-induced plasma proteome changes only on samples collected at 60 min after injury. Moreover, because of the low throughput, plasma samples would have been pooled from different animals in the same experimental group. We would have then used antibody-based assays to confirm and evaluate the correlation of these changes with trauma intensity and time of collection after injury.  Potential biomarkers detectable only at times different than 60 min post-injury would have been missed.

As an alternative to metabolic labeling and ICAT, the "subtractive proteomics" approach does not involve pre-labeling or tagging samples. Relative differences in expression are quantitated in terms of differences in the number of tryptic peptides that can be identified for a specific protein following tandem mass spectrometry analysis. Changes in this number appear to roughly correlate with changes in concentrations of the related protein [Sanders *et al*, Mol Cell Biol.

2002 Jul;22(13):4723-38, Rappsilber *et al*, Genome Res. 2002 Aug;12(8):1231-45]. Though less accurately quantitative than comparison of tagged peptides, the subtractive proteomics approach appears to be adequate for studies like this, where the scope of initial mass spectrometry-based proteomic evaluations is to provide a preliminary "hit-list' of potential predictors that will then need to be confirmed and more accurately quantitated by antibody-based assays. Most importantly, the differential proteomics approach allows for a much higher throughput than that allowed by the other approaches. Adoption of this approach has allowed us to plan the preliminary proteomic analyses to be carried out on individual plasma samples (four from each involved experimental group) collected not just at 60 min but at different times after injury, as described in the following section.

*Objective 3    Preliminary identification of proteins differentially expressed in plasma from rat models of traumatic brain injury.*

The original plan called for the sera collected at 60 min after either form of TBI to be used for the identification of serum proteomic changes following TBI. In order to allow for proteomic analysis, as originally envisioned, the different samples from each experimental group needed to be pooled. The achievement of Objective 2 and adoption of a proteomic technology with higher throughput has allowed us to modify the experimental design. The new "Subtractive Proteomics" approach has allowed us to test single plasma samples (not pooled) from 104 rats, i.e. from 4 rats in each of 26 experimental groups (as defined by TBI treatment and collection time and described in Table 1).

**MATERIAL AND METHODS**

**Animals**

One hundred seventy nine Sprague-Dawley male rats (Taconic, Germantown, NY) weighing 250-300 g have been used. Rats were housed in the facilities of the Laboratory Animal Medicine (LAM), at the Uniformed Services University of the Health Sciences (USUHS), with *ad libitum* access to food and water. Excluding the ones used for technical refinement, 139 rats have been used for actual experiments. Experimental group allocation of these rats is summarized in Table 1. Twenty-six experimental conditions were tested as defined by TBI treatment and time of collection: (1) naïve, n = 5; (2) anesthesia only (ANS) at 15 min, n = 5; (3) ANS at 60 min, n = 5; (4) ANS at 6 hours, n = 5; (5) ANS at 24 hours, n = 5; (6) ANS at 72 hours, n = 5; (7) lateral fluid percussion injury at 2.5 atm (FPI-III) at 15 min, n = 5; (8) FPI-III at 60 min, n = 6; (9) FPI-III at 6 hours, n = 5; (10) FPI-III at 24 hours, n = 6; (11) FPI-III at 72 hours, n = 5; (12) Surgical

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preparation for FPI without TBI (FPI-0), at 15 min, n = 5; (13) FPI-0 at 60 min, n = 5; (14) FPI-0 at 6 hr, n = 5; (15) FPI-0 at 24 hour, n = 5; (16) FPI-0 at 72 hour, n = 6; (17) Penetrating Ballistic Injury at 7.5% volume (FPI-III) at 15 min, n = 6; (18) FPI-III at 60 min, n = 5; (19) FPI-III at 6 hour, n = 7; (20) FPI-III at 24 hour, n = 6; (21) FPI-III at 72 hour, n = 6; (22) Surgical preparation for PBI without TBI (PBI-0) at 15 min, n = 5; (23) PBI-0 at 1 hour, n = 5; (24) PBI-0 at 6 hour, n = 5; (25) PBI-0 at 24 hour, n = 5; (26) PBI-0 at 72 hour, n = 5. At least 3 days were given to allow each animal to equilibrate to their new surroundings after delivery.

**Surgery (application of traumatic brain injury)**

All surgeries were performed using aseptic technique. Animals were checked for pain/distress immediately prior to anesthesia/surgery. Naïve animals were not exposed to anesthesia or surgery until time of biosample collection. All other animals were anesthetized with 3.5% isoflurane in oxygen and maintained with 1-3% isoflurane. Body temperature was maintained with a heating pad. For the anesthesia control groups, animals were maintained under anesthesia in the induction chamber for approximately 1 hour (average duration of the surgical procedure). Anesthesia was discontinued and the animal transferred to a recovery area. Once animal was ambulatory, it was transferred to its home cage, where it was allowed to behave freely with *ad libitum* access to food and water. All animals in the 24 hour and 72 hour groups were returned to LAM within 12 hours of leaving. Pain/distress checks were performed at 3, 6, 12, 24, 36, 48, 60, and 72 hours post-injury.

*Fluid Percussion Injury (FPI)*

For animals in the surgical control and injury groups, a midline incision was made to expose the dorsal surface of the skull, and a 5 mm burr hole drilled over the right cerebral cortex at 2.5 mm caudal and 2.5 mm right of bregma. A modified cannula hub was fitted to the burr hole and glued in place. After carefully attaching the fluid percussion device (MCV Bioengineering, Richmond, VA), a fluid percussion pulse was either not given (for the surgical controls) or administered at 0.5, 1.5, or 2.5 atm. The hub was immediately removed and the incision closed with wound clips. The animal was recovered as above.

*Penetrating Ballistic Injury (PBI)*

For animals in the surgical control and injury groups, a midline incision was made to expose the dorsal surface of the skull and the occipital protuberance. A 3 mm burr hole was drilled through the occipital plate at 4 mm ventral and 2 mm right of midline. The animals in the surgical control group remained on anesthesia for the remainder of the hour prior to wound closure and recovery. Animals in the PBI-I, -II, and -III groups were injured using the PBI device (Dragonfly R&D, Ridgeley, WV). An inflatable probe (Dragonfly R&D, Ridgeley, WV) was inserted 15 mm through the right hemisphere and either not inflated (PBI-0) or inflated to create a 5% or 7.5% brain volume injury. The probe was immediately removed and the wound closed with wound clips. The animal was recovered as above.

**Neurobehavioral evaluations**

Prior to biosample collection, animals in the 24 hour and 72 hour groups were evaluated using the Neurologic Severity Score (NSS), NIH Stroke Severity Score (NIH), and Neurobehavioral Scale (NBS). The criteria used to obtain the scores are detailed in Tables 2 and 3. As an additional endpoint, we also recorded the time needed for each animal to "right up" following the interruption of anesthesia ("righting reflex").

**Biosample collection and processing**

At the time of terminal biosample collection, animals were anesthetized with 3.5% isoflurane in oxygen and maintained at 1-3% isoflurane.

*CSF collection and processing*

After placement in the stereotactic apparatus using blunt-tip ear bars only, a midline incision was made and the muscle dissected away to expose the atlanto-occipital membrane. The area was kept free of blood until puncture of the membrane to collect CSF. Ten minutes were maintained between time of induction and time of CSF collection. CSF was collected using a 25G permanently attached needle on a 1cc insulin syringe (Monoject, lot # 524416) into sterile 0.6 ml tubes (FisherBrand, lot # 451706) and kept at room temperature. CSF was spun at 15 minutes post-collection at 1300 x g for 10 min at $22^o$C. The supernatant was spun at 35 minutes at 12,000 x g for 15 min at $22^o$C in sterile 0.6 ml tubes (FisherBrand, lot # 451706). The supernatant was placed in a liquid nitrogen storage tank at 55 minutes post-collection in 1 ml cryogenic vials (Nalgene System-100, lot # 554872).

After collection of CSF, animals were removed from the stereotax, placed supine, and anesthesia increased to 3-5% isoflurane. A midline incision was made over the ribs and abdomen, and the abdominal muscles were cut laterally below the diaphragm.

*Blood collection and processing*

At 5 minutes post-CSF collection, blood was collected transcardially after cutting through the diaphragm/ribs to expose the heart. Blood was collected in a 4ml EDTA vacutainer (BD, lot # 5157524) using a 20G blood collection needle (Monoject, lot # 216025) and kept at room temperature. At 10 minutes post-collection, the blood was spun at 1321 x g for 10 min at $22^o$C. Plasma was transferred into two 2 ml tubes (FisherBrand, lot # 534388) and spun at 12,000 x g for 15 min at $22^o$C. The buffy coat was transferred into a 15 ml tube (Phenix, lot # 3218P) containing 6 ml of RPMI 1640 (Biosource, lot # 604085) and resuspended. The buffy coat mixture was then carefully transferred into a 15 ml tube (Phenix, lot # 3218P) containing 5 ml of Histopaque-1077 (Sigma, lot # 065K6208) and spun at 800 x g for 15 minutes at room temperature. Of the original blood separation, 10 µl of the red blood cell (RBC) pellet was transferred into a 15 ml tube (Phenix, lot # 3218P) containing 10 ml of RPMI 1640 (Biosource, lot # 604085), resuspended, and kept at room temperature. Plasma supernatant was transferred into 1 ml cryogenic vials (Nalgene System-100, lot # 554872) and stored in a liquid nitrogen storage tank at 50 minutes post-blood collection. At 60 minutes after blood collection, the mononuclear cell (MNC) layer from the Histopaque preparation is transferred into a 15 ml tube (Phenix, lot # 3218P) containing 10 ml of RPMI 1640 (Biosource, lot # 604085), resuspended, and spun along with the RBC suspension at 460 x g for 10 min at $22^o$C. The pellets were resuspended in 2 ml freezing medium (10% DMSO; 10% calf serum; 80% RPMI 1640, Biosource, lot # 604085). The RBC and MNC suspensions were split into 1 ml cryogenic vials (Nalgene System-100, lot # 554872). By 80 minutes after blood collection, the RBC and MNC suspensions were placed in a styrofoam "jacket" at -80$^o$C overnight before being transferred into the liquid nitrogen storage tank.

*Brain collection and processing*

Immediately after blood collection, animals were either fixed or the brains collected fresh. For fixation, animals were transcardially perfused with PBS until the fluid ran clear from the cut right atrium, then perfused with 10% formalin until the body became stiff. Brains were dissected out and kept at 4$^o$C in 10% formalin overnight, in 10% sucrose overnight, in 20% sucrose for 2-3 days, in 30% sucrose for 2-3 days, then frozen in OCT (Optimum Cutting temperature) embedding compound using isopentane/dry ice. Frozen brains are stored at -80$^o$C. Brains collected fresh began the freezing process in OCT Compound using isopentane/dry ice 2-3 minutes after blood collection.

**Proteomic analysis of plasma samples**

*Phase I: High abundant protein depletion*

High-abundant proteins we first *depleted* from rat plasma samples using Agilent's MARS (Multiple Affinity Removal System) mouse cartridges. This system utilizes an immunoaffinity cartridge packed with antibody-modified resin. The cartridge is specifically designed to remove three highly-abundant proteins (albumin, IgG and transferrin) simultaneously from serum or plasma. An enriched pool of low-abundant proteins was then collected and pooled from several flow-through fractions. Agilent's 5000 Da molecular weight cut off (MWCO) spin concentrators were then used for desalting and concentrating the low-abundant proteins.

*Quality control* for this phase was achieved by using every fifth rat plasma sample to test the efficiency of the MARS cartridges. An aliquot of each flow-through fraction and an aliquot of fractions containing high abundant proteins is removed and run on 4-12% Bis-Tris NuPAGE gels.

*Phase II: protein digestion*

Protein digestion was accomplished through the use of sequencing grade modified trypsin (Promega). Resultant peptides were subjected to solid phase extraction (SPE) using 3M$^{TM}$ Empore$^{TM}$ High Performance Extraction Disk Cartridges (3M Bioanalytical Technologies, St. Paul, MN). This procedure not only concentrates resultant peptides, but also removes or minimizes substances that may interfere with downstream sample processing.

For *quality control* purposes, complete digestion was verified by removing an aliquot of each trypsin-digested plasma sample and running it on a 4-12% Bis-Tris NuPAGE gel. BCA protein assays (Pierce, Rockford, IL) were performed on all rat plasma samples both prior to trypsin digestion and prior to SCX processing.

*Phase III: Strong Cation Exchange (SCX) Fractionation*

SCX chromatography was used to reduce sample complexity prior to tandem mass spectrometry. Briefly, strong cation exchange chromatography was performed using a HP 1100 LC system (Agilent Technologies, Palo Alto, CA) equipped with a polysulfoethyl A column (1.0 x 150mm, 5μm, 300Å pore size, PolyLC, Inc.). An ammonium formate, pH 3.0/acetonitrile multi-step gradient was used to elute peptides from the column at a flow rate of 50 μl/min. Peptide separation was monitored by fluorescence (266/340 nm). A total of 96 fractions are collected which were subsequently pooled into 10 fractions, lyophilized and re-suspended in 0.1% trifluoroacetic acid (TFA) for tandem mass spectrometry.

*Phase IV: Tandem Mass Spectrometry*

A total of 1040 samples were been prepared and analyzed by tandem mass spectrometry (104 plasma samples x 10 fractions/sample). µRPLC-MS/MS was performed using an Agilent 1100 capillary LC system (Agilent Technologies, Palo Alto, CA) coupled online to a linear ion trap (LIT) mass spectrometer (LTQ, Thermo Electron, Waltham, MA, USA). Separations were performed using 75µm i.d. x 360 µm o.d. x 10 cm long fused silica capillary columns (Polymicro Technologies, Phoenix, AZ) slurry-packed in house with 3 µm, 300Å pore size C-18 silica-bonded stationary phase (Vydac, Hysperia, CA).

The mass spectrometer was operated in a data-dependent MS/MS mode with each full MS scan being followed by seven MS/MS scans where the seven most abundant molecular ions were dynamically selected for collision-induced dissociation (CID) using a normalized collision energy of 35%.

*Phase V: Bioinformatics*

Tandem mass spectra were searched against the UniProt rat proteomic database (10-26-05 release) from the European Bioinformatics Institute (www.ebi.ac.uk/integr8) with SEQUEST® (Thermo Electron). For a fully tryptic peptide to be considered legitimately identified, it had to achieve stringent charge state and proteolytic cleavage-dependent cross correlation ($X_{corr}$) scores of 1.9 for $[M^+H]^{1+}$ , 2.2 for $[M^+2H]^{2+}$ , and 3.1 for $[M^+3H]^{3+}$, and a minimum delta correlation (∆Cn) of 0.08. SEQUEST results were further filtered and analyzed using software developed in-house.

**Analysis of proteomic data**

To identify proteins differentially represented in the plasma of TBI and control rats, we compared the mean number of unique peptide sequences detected per each protein. Prioritization of protein "hits" was based on the statistical significance of observed differences. This was a two-step process with a first t test carried out on the differences in number of unique peptides between each TBI experimental condition (as defined kind of TBI and time of collection after injury) and its own surgical sham (e.g., FPI-III *vs* FPI-0), for all proteins detected (overall, 10217 proteins were identified by at least two unique peptides). This first statistical test compared the results in plasma from 4 rats in each of the two experimental groups. Only proteins for which the level of statistical significance for the observed difference is $p \leq 0.2$ were selected for the second statistical test. The second t test compared the mean number of unique peptides for each of the selected proteins between each TBI conditions (as defined by kind of TBI and time of collection – corresponding to the mean of 4 samples) and all control conditions (including all the different control conditions at the different collection times – corresponding to the mean of 64 samples) . The result of this two-step analysis is represented by tables, one for each TBI conditions, in which plasma proteins are ranked according to the statistical significance of the observed differences (*i.e.*, in order of ascending *p* value).

**RESULTS**

As described in the application for this grant, we designed this as a 3-year study, but we applied and obtained funding only for year 1, with the idea to apply additional funding based on year 1 results. What follows describes these results.

**Objective 1**

Application of traumatic brain injury, neurobehavioral testing and collection of biofluids (plasma, CSF, mononuclear blood cells and erythrocytes) and brains have been carried out on 138 rats, including at least 5 rats for each of 26 experimental conditions, as defined by kind of injury, related control conditions and time tissue/plasma collection after injury. Experimental conditions and the number of animals in each experimental group are summarized in Table 1. The experimental conditions include all 3 control conditions (surgical preparation without actual application of TBI, anesthesia only, and baseline control), and the lateral fluid percussion (FPI) and Penetrating Ballistic (PBI) forms of TBI at one of 3 planned intensities (2.5 atm and 7.5% volume, respectively). Application of two additional intensities is part of the work proposed for year 2 and 3 of the study.

Results of the procedures are summarized in Table 4. For each rat that was used, the table details the study identifier, experimental group, amount of plasma and CSF collected, whether red blood cells (RBC) and mononuclear cells (MNC) were collected, whether the brain was collected fresh or fixed, the Neuroscores (NSS, NBS and NIH) and righting reflex time (time needed for the animal to right itself onto all four paws, following interruption of anesthesia).

We observed that CSF collection is achievable at 6 and more hours after brain injury, but not 1 hour or at shorter times after injury. Consequently, this medium could not be used as a source of early biomarkers of TBI (1 hour or less after injury).

Brains will be used in year 2 and 3 of this study for characterization of the lateral fluid percussion *vs* penetrating ballistic penetrating models of traumatic brain injury by histochemistry and immunohistochemistry.

CSF and mononuclear and red blood cells may be used in further and separate studies on proteomic and/or transcriptomic biomarkers from these sources.

Functional scores have been collected both for characterization purposes and to evaluate their correlation with possible plasma proteomic changes. The righting reflex times and the three neurobehavioral scores demonstrate a significant effect of both kinds of TBI. The scores also suggest a more intense effect of PBI as compared to FPI.

Overall, we believe Objective 1 for the first year of this study has been fully met.

**Objective 2**

As described in the Experimental Design section of this report, Objective 2 has been met by the adoption of a "differential proteomics approach" to the identification of proteins differentially expressed in plasma of TBI rats. The higher throughput of this approach has allowed us to greatly expand the number of plasma samples that can be subjected to proteomic analysis as part of Objective 3 for year 1.

**Objective 3**

Plasma samples from 104 animals (4 rats for each of 26 treatment groups described in the Experimental Design section of this report and in Table 1) have been delivered to the proteomic lab directed by Dr. Veenstra.

Proteomic analysis has been completed. This is a large study and, even with the greatly increased throughput afforded by the differential proteomics approach to data analysis, processing and analyzing these many samples and the resulting data has represented a formidable task (as detailed below), that has taken considerable more time than originally planned.

As described in the *Material and Methods* section of this report, proteins differentially represented in plasma of TBI rats (representing potential biomarkers of TBI) have been identified by a subtractive proteomic approach, whereas the number of unique peptides detected for each protein represents a measure of its abundance. Overall, 10217 proteins were identified by at least two unique peptides across the 104 plasma samples that were tested. On average, between 1500 and 2500 proteins were identified within each plasma sample. To identify the best

potential biomarkers of TBI, proteins were ranked according to the statistical significance of observed differences (see *Material and Methods* section).

Fig.1a (FPI) and Fig 1b (PBI) provide a graphical representation of the number of differentially represented plasma proteins in relation to time of collection after injury, using different cut-offs for statistical significance of observed differences. The most striking aspect of these results is the difference between the FPI and PBI model. The results suggest a much more dramatic impact of the penetrating ballistic injury as compared to the lateral fluid percussion injury. This is consistent with the results of neurobehavioral evaluations showing a more profound effect of PBI. These results also show a correlation between protein changes and time after injury. This correlation is particularly evident for PBI, where a first peak is observed at 15 min after injury, followed by a bigger peak at 6 hours. It could be speculated that the first peak may correspond to the immediate release of tissue factors due to the primary injury, while the second wave may represent secondary injury.

Given the amount of data involved (including more than 10,000 proteins overall), only the proteins for which the most significant differences were observed are reported here. Tables 5a-e report proteins, for which the statistical significance of FPI-related differences corresponds to $p<10^{-3}$. Table a-e report the equivalent results for PBI, but with a lower cut-off ($p<10^{-4}$), given the much greater depth of differences observed. In each table, the mean number of unique peptides being compared is color coded in blue, the p value in red, and the proteins known to be associated with nervous tissue are marked bold.

The results represented in Tables 5 and 6 show most of the proteins were identified at the lowest level of detection (2-4 unique peptides across 4 plasma samples). This stresses the need for more sensitive, antibody-based assays to verify and better the observed results. Given the amount of proteins involved, only a small fraction of these can be carried on to further verification and validation studies. Tables 7a-j represent a broad selection from the proteins listed in Tables 5 and 6, based on a combination of criteria: biological significance (e.g., known expression in the central nervous), statistical significance of observed differences, identification of significant differences across different times of collection, availability of antibody based assays. We selected 3-6 proteins for each TBI model/collection time though there are differences in statistical significance for proteins identified in different experimental groups. Some of these proteins (butyrophilin-like protein 2 and testicular cell adhesion molecule 1) are among the most

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significant across different TBI collection times. Consequently, they have been selected even if their association with the central nervous tissue is presently unknown. Interestingly, testicular cell adhesion molecule 1 is member of a family of cell adhesion molecules including at least one (ICAM-1) whose intestinal levels are known to increase in response to TBI) [Hang *et al.*, World J Gastroenterol. 2005: 11(8): 1149-54]. In tables 7a-j, a graph is associated to each of the selected proteins representing the observed behavior through time.

Some of the selected proteins are of particular interest for their potentially high biologically significance. For example, *syntaxin-1A* ($p = 2.3$ x $10^{-9}$) appears to be specifically expressed in neurons [Yi et al., J Neurotrauma 2006, 23(1): 86-96]]. *Ischemia related factor vof-16* ($p = 4.3$ x $10^{-11}$) is a scarcely known factor which has been first identified as upregulated in hippocampus and brain cortex following brain ischemia [Todha et al, Biol.Pharm.Bull. 2004, 27(8): 1228-35]. Proenkephalin B ($p = 4.3$ x $10^{-11}$) is a known precursor of several opioid peptides with important functions in the brain [Henry *et al*, Exp Neurol 2003, 183(2):458-68; Arendt et al Brain Res. 1985, 345(2):230-7]

## CONCLUSIONS

**Objectives 1-3 for year 1 of this multi-year Project have been fully met**. TBI has been applied and functional outcome data and biosamples collected from more than half of the animals minimally required for this 3-year study. We have adopted an approach to proteomic analysis that has allowed us to vastly expand the scope of the third Objective for year 1, **allowing us to screen not only for potential early, but also for later biomarkers of TBI**. We now have a list of potential biomarkers on closed-head (FPI model) and penetrating ballistic injury (PBI) at different times following injury. Further funding will be needed both to expand the study to include both a verification and better quantitation, and the evaluation of the trauma intensity-dependence of the observed plasma proteome changes.

**TABLE 1    Number of rats used for each experimental conditions tested in the course of this study**

| | | Naive[a] | ANS only[b] | Lateral Fluid Percussion Model | | Ballistic Penetrating Model | |
|---|---|---|---|---|---|---|---|
| | | | | 0 sham | III 2.5atm | 0 sham | III 7.5% |
| Biosample collection time (time since injury) | No time ref[a] | 5 | -- | -- | -- | -- | -- |
| | 15 min | -- | 5 | 5 | 5 | 5 | 6 |
| | 60 min | -- | 5 | 5 | 6 | 5 | 5 |
| | 6 hours | -- | 5 | 5 | 5 | 5 | 7 |
| | 24 hours | -- | 5 | 5 | 6 | 5 | 6 |
| | 72 hours | -- | 5 | 6 | 5 | 5 | 6 |

[a] Baseline control group (animals receive no injury, surgery,  nor anesthesia). [b] Animals treated with anesthesia only

**TABLE 2    Neurologic Severity Score (NSS)**

| Neurologic Severity Score (NSS) | Points |
| --- | --- |
| Inability to exit from a circle (50 cm in diameter) when left in its center | |
|     Within 30 min | 1 |
|     Within 60 min | 1 |
|     At >60 min | 1 |
| Loss of righting reflex | |
|     For 20 min | 1 |
|     For 40 min | 1 |
|     For 60 min | 1 |
| Hemiplegia-inability of the rat to resist forced changes in position | 1 |
| Flexion of hindlimb when raised by the tail | 1 |
| Inability to walk straight when placed on the floor | 1 |
| Inability to move | 1 |
| Reflexes | |
|     Startle reflex | 1 |
|     Pinna reflex | 1 |
| Clinical grade | |
|     Loss of seeking behavior | 1 |
| Prostration | 1 |
| Limb Reflexes | |
|   Loss of placing reflexes | |
|     Forelimbs left | 1 |
|     Forelimbs right | 1 |
|     Hindlimbs left | 1 |
|     Hindlimbs right | 1 |
| Functional tests | |
|     Failure in beam balancing task (1 .5 cm wide) | |
|       For 20 s | 1 |
|       For 40 s | 1 |
|       For 60 s | 1 |
|     Failure in beam-walking task | |
|       8.5 cm wide | 1 |
|       5.0 cm wide | 1 |
|       2.5 cm wide | 1 |
| TOTAL POINTS | 24 |

**TABLE 3   Neurobehavioral Scale and NIH Stroke Severity Score**

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**Neurobehavioral Scale**

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*Each category is scored on a scale of 0-4 with 0 being non-functional and 4 being normal.*

Forelimb Flexion upon suspension by tail
Decrease in resistance to lateral pulsion
Circling behavior upon spontaneous ambulation
Ability to stand on an inclined plane
Open Field Activity/ Exploratory Behavior

TOTAL POINTS      20

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**NIH Stroke Severity Score**

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Based on observed deficits, a functional grade is assessed:
      Grade 0: Normal rat
      Grade 1: Lethargy
      Grade 2: Clear signs of paresis in at least one limb but able to walk
      Grade 3: Severe paresis/paralysis, unable to walk
      Grade 4: Dead

---

| Sample ID | Expt Group | Total Amount of Plasma (ml) | Total Amount of CSF (µl) | brain | RBC in storage | MNC in storage | NSS | NIH | NBS | righting time (min) |
|---|---|---|---|---|---|---|---|---|---|---|
| AA123 | naïve | 2.04 | 140 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA148 | naïve | 1.9 | 50 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA164 | naïve | 2.05 | 120 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA268 | naïve | 1.97 | 120 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AB027 | naïve | 2 | 120 | fresh frozen | + | + | n/a | n/a | n/a | n/a |
| AA084 | A 15min | 1.94 | 0 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA194 | A 15min | 2 | 90 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA260 | A 15min | 2 | 80 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AB017 | A 15min | * | 80 | fresh frozen | + | + | n/a | n/a | n/a | n/a |
| AB018 | A 15min | 1.75 | 90 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA131 | A 60min | 2 | 80 | fixed frozen | + | + | n/a | n/a | n/a | 4 |
| AA173 | A 60min | 1.85 | 60 | fixed frozen | + | + | n/a | n/a | n/a | * |
| AA206 | A 60min | 1.9 | 110 | fixed frozen | + | + | n/a | n/a | n/a | * |
| AA269 | A 60min | 2 | 110 | fixed frozen | + | + | n/a | n/a | n/a | 2 |
| AB035 | A 60min | 2.1 | 160 | fresh frozen | + | + | n/a | n/a | n/a | 3 |
| | | | | | | **Mean =** | n/a | n/a | n/a | 3 |
| AA129 | A 6hr | 1.8 | 140 | fixed frozen | + | + | 0 | 0 | 20 | NR |
| AA161 | A 6hr | 1.8 | 140 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AA215 | A 6hr | 1.9 | 140 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AA259 | A 6hr | 1.92 | 120 | fixed frozen | + | + | 0 | 0 | 20 | 2 |
| AA290 | A 6hr | 1.8 | 120 | fresh frozen | + | + | 0 | 0 | 20 | 2 |
| | | | | | | **Mean =** | 0 | 0 | 20 | 2.5 |
| AA070 | A 24hr | 1.86 | 120 | fixed frozen | + | + | 0 | 0 | 20 | 2 |
| AA163 | A 24hr | 1.94 | 80 | fixed frozen | + | + | 0 | 0 | 20 | 2 |
| AA192 | A 24hr | 1.92 | 140 | fixed frozen | + | + | 0 | 0 | 20 | * |
| AA233 | A 24hr | 2 | 100 | fixed frozen | + | + | 0 | 0 | 20 | 5 |
| AB005 | A 24hr | 1.98 | 75 | fresh frozen | + | + | 0 | 0 | 20 | 1 |
| | | | | | | **Mean =** | 0 | 0 | 20 | 2.5 |
| AA072 | A 72hr | 2 | 110 | fixed frozen | + | + | 0 | 0 | 20 | 2 |
| AA190 | A 72hr | 1.9 | 130 | fixed frozen | + | + | 0 | 0 | 20 | * |
| AA234 | A 72hr | 2 | 110 | fixed frozen | + | + | 0 | 0 | 20 | * |
| AA266 | A 72hr | 1.9 | 100 | fixed frozen | + | + | 0 | 0 | 20 | NR |
| AB003 | A 72hr | 1.75 | 100 | fresh frozen | + | + | 0 | 0 | 20 | * |
| | | | | | | **Mean =** | 0 | 0 | 20 | 2 |
| | | | | | **Mean for All ANS Animals =** | | 0 | 0 | 20 | 2.6 |

| Sample ID | Expt Group | Total Amount of Plasma (ml) | Total Amount of CSF (µl) | brain | RBC in storage | MNC in storage | NSS | NIH | NBS | righting time (min) |
|---|---|---|---|---|---|---|---|---|---|---|
| AA036 | FPI 0 15min | 1.76 | 120 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA130 | FPI 0 15min | 2 | 80 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA168 | FPI 0 15min | 1.94 | 0 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA196 | FPI 0 15min | 2 | 100 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA267 | FPI 0 15min | 1.92 | 120 | fresh frozen | + | + | n/a | n/a | n/a | n/a |
| | | | | | | | | | | |
| AA124 | FPI 0 60min | 2 | 140 | fixed frozen | + | + | n/a | n/a | n/a | 2 |
| AA154 | FPI 0 60min | 1.1 | 90 | fixed frozen | + | + | n/a | n/a | n/a | 4 |
| AA174 | FPI 0 60min | 2 | 100 | fixed frozen | + | + | n/a | n/a | n/a | 2 |
| AA236 | FPI 0 60min | 2.04 | 76 | fixed frozen | + | + | n/a | n/a | n/a | 7 |
| AB034 | FPI 0 60min | 2 | 96 | fresh frozen | + | + | n/a | n/a | n/a | 2 |
| | | | | | | | **Mean =** | n/a | n/a | n/a | 3.4 |
| AA122 | FPI 0 6hr | 1.95 | 140 | fixed frozen | + | + | 0 | 0 | 20 | 4 |
| AA155 | FPI 0 6hr | 2 | 100 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AA195 | FPI 0 6hr | 2 | 180 | fixed frozen | + | + | 0 | 0 | 20 | 4 |
| AA258 | FPI 0 6hr | 2 | 140 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AA281 | FPI 0 6hr | 2.1 | 90 | fresh frozen | + | + | 0 | 0 | 20 | 2 |
| | | | | | | | **Mean =** | 0 | 0 | 20 | 3.2 |
| AA035 | FPI 0 24hr | 1.96 | 110 | fixed frozen | + | + | 0 | 0 | 20 | 5 |
| AA045 | FPI 0 24hr | 1.6 | 150 | fixed frozen | + | + | 0 | 0 | 20 | NR |
| AA076 | FPI 0 24hr | 1.88 | 120 | fixed frozen | + | + | 0 | 0 | 20 | NR |
| AA273 | FPI 0 24hr | 2.1 | 160 | fixed frozen | + | + | 1 | 0 | 20 | 2 |
| AB051 | FPI 0 24hr | 2 | 0 | fresh frozen | + | + | 0 | 0 | 20 | 4 |
| | | | | | | | **Mean =** | 0.2 | 0 | 20 | 3.7 |
| AA019 | FPI 0 72hr | 1.66 | 85 | fixed frozen | + | + | 0 | 0 | 18 | 4 |
| AA068 | FPI 0 72hr | 1.76 | 80 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AA193 | FPI 0 72hr | 2 | 140 | fixed frozen | + | + | 3 | 0 | 16 | 6 |
| AB011 | FPI 0 72hr | 1.9 | 60 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AB004 | FPI 0 72hr* | 2.1 | 70 | fresh frozen | + | + | 0 | 0 | 20 | 3 |
| | | | | | | | **Mean =** | 0.6 | 0 | 18.8 | 3.8 |
| | | | | | | **Mean for All FPI-0 Animals =** | | 0.3 | 0 | 19.6 | 3.5 |

| Sample ID | Expt Group | Total Amount of Plasma (ml) | Total Amount of CSF (µl) | brain | RBC in storage | MNC in storage | NSS | NIH | NBS | righting time (min) |
|---|---|---|---|---|---|---|---|---|---|---|
| AA024 | FPI III 15min | 1.72 | 0 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA082 | FPI III 15min | 1.8 | 0 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA113 | FPI III 15min | 1.8 | 0 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA208 | FPI III 15min | 1.95 | 0 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AA292 | FPI III 15min | 2 | 0 | fresh frozen | + | + | n/a | n/a | n/a | n/a |
| | | | | | | | | | | |
| AA149 | FPI III 60min | 0.19 | 50 | fixed frozen | + | + | n/a | n/a | n/a | 10 |
| AA162 | FPI III 60min | 1.9 | 0 | fixed frozen | + | + | n/a | n/a | n/a | 11 |
| AA169 | FPI III 60min | 1.94 | 0 | fixed frozen | + | + | n/a | n/a | n/a | 7 |
| AA282 | FPI III 60min | 1.88 | 0 | fixed frozen | + | + | n/a | n/a | n/a | 10 |
| AB050 | FPI III 60min | 1.94 | 0 | fresh frozen | + | + | n/a | n/a | n/a | 10 |
| | | | | | | **Mean =** | n/a | n/a | n/a | 9.6 |
| | | | | **p-value (T test FPI-0 vs FPI-III at 1 hour) =** | | | #DIV/0! | #DIV/0! | #DIV/0! | 0.001 |
| | | | | | | | | | | |
| AA112 | FPI III 6hr | 1.8 | 80 | fixed frozen | + | + | 0 | 0 | 20 | 5 |
| AA147 | FPI III 6hr | 1.95 | 0 | fixed frozen | + | + | 2 | 1 | 17 | 10 |
| AA172 | FPI III 6hr | 1.92 | 90 | fixed frozen | + | + | 4 | 1 | 17 | 9 |
| AA207 | FPI III 6hr | 2 | 0 | fixed frozen | + | + | 8 | 1 | 15 | 8 |
| AA271 | FPI III 6hr | 1.84 | 50 | fresh frozen | + | + | 4 | 1 | 17 | 8 |
| | | | | | | **Mean =** | 3.6 | 0.8 | 17.2 | 8 |
| | | | | **p-value (T test FPI-0 vs FPI-III at 6 hours) =** | | | 0.0265 | 0.0039 | 0.0081 | 0.0008 |
| | | | | | | | | | | |
| AA020 | FPI III 24hr | 1.8 | 80 | fixed frozen | + | + | 0 | 0 | 20 | 6 |
| AA071 | FPI III 24hr | unk | 0 | fixed frozen | + | + | 0 | 0 | 18 | 8 |
| AA167 | FPI III 24hr | 2 | 25 | fixed frozen | + | + | 2 | 1 | 15 | 13 |
| AA235 | FPI III 24hr | 2 | 40 | fixed frozen | + | + | 3 | 1 | 15 | 21 |
| AA272 | FPI III 24hr | 1.9 | 40 | fresh frozen | + | + | 2 | 0 | 18 | 6 |
| AC-010 | FPI III 24hr* | 1.9 | 130 | fresh frozen | + | + | 0 | 0 | 20 | 16 |
| | | | | | | **Mean =** | 1.2 | 0.3 | 17.7 | 11.7 |
| | | | | **p-value (T test FPI-0 vs FPI-III at 24 hours) =** | | | 0.157 | 0.186 | 0.047 | 0.066 |
| | | | | | | | | | | |
| AA034 | FPI III 72hr | unk | 0 | fixed frozen | + | + | 0 | 0 | 20 | 7 |
| AA069 | FPI III 72hr | 1.96 | 80 | fixed frozen | + | + | 0 | 0 | 20 | 6 |
| AA191 | FPI III 72hr | 2 | 100 | fixed frozen | + | + | 0 | 0 | 19 | 18 |
| AA232 | FPI III 72hr | 2.1 | 50 | fixed frozen | + | + | 1 | 0 | 18 | 10 |
| AB006 | FPI III 72hr* | 1.8 | 120 | fresh frozen | + | + | 0 | 0 | 19 | 11 |
| | | | | | | **Mean =** | 0.2 | 0 | 19.2 | 10.4 |
| | | | | **p-value (T test FPI-0 vs FPI-III at 72 hours) =** | | | 0.545 | #DIV/0! | 0.663 | 0.017 |
| | | | | | | | | | | |
| | | | | **Mean for All FPI-III Animals =** | | | 1.6 | 0.4 | 18 | 10 |
| | | | | **p-value (T test All FPI-0 vs All FPI-III) =** | | | 0.035 | 0.007 | 0.008 | 1.84E-07 |

(continued ….)

## Table 4: Biosamples and neurobehavioral scores (Part 4 of 5)

| Sample ID | Expt Group | Total Amount of Plasma (ml) | Total Amount of CSF (µl) | brain | RBC in storage | MNC in storage | NSS | NIH | NBS | righting time (min) |
|---|---|---|---|---|---|---|---|---|---|---|
| AB060 | PBI 0 15min | 1.9 | 80 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AB095 | PBI 0 15min | 1.85 | 80 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AB167 | PBI 0 15min | 2 | 70 | fixed frozen | - | - | n/a | n/a | n/a | n/a |
| AB168 | PBI 0 15min | 1.95 | 60 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| | | | | | | | | | | |
| AB098 | PBI 0 60min | 2 | 100 | fixed frozen | + | + | n/a | n/a | n/a | 2 |
| AB149 | PBI 0 60min | 2.05 | 124 | fixed frozen | + | + | n/a | n/a | n/a | 5 |
| AB182 | PBI 0 60min | 1.95 | 55 | fixed frozen | + | + | n/a | n/a | n/a | 6 |
| AB231 | PBI 0 60min | 2 | 110 | fresh frozen | + | + | n/a | n/a | n/a | 3 |
| AB253 | PBI 0 60min | 2 | 57 | fixed frozen | + | + | n/a | n/a | n/a | 14 |
| | | | | | | **Mean =** | n/a | n/a | n/a | 6 |
| | | | | | | | | | | |
| AB185 | PBI 0 6hr | 1.97 | 140 | fixed frozen | + | + | n/a | n/a | n/a | |
| AB094 | PBI 0 6hr | 1.95 | 70 | fixed frozen | + | + | 0 | 0 | 20 | 4 |
| AB116 | PBI 0 6hr | 2.1 | 140 | fixed frozen | + | + | 0 | 0 | 20 | 5 |
| AB143 | PBI 0 6hr | 2 | 70 | fixed frozen | + | + | 0 | 0 | 18 | 5 |
| AB184 | PBI 0 6hr | 2 | 85 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AB230 | PBI 0 6hr | 2.05 | 115 | fresh frozen | + | + | 0 | 0 | 20 | 9 |
| | | | | | | **Mean =** | 0 | 0 | 19.6 | 5.2 |
| | | | | | | | | | | |
| AB090 | PBI 0 24hr | 2.15 | 90 | fixed frozen | + | + | 0 | 0 | 20 | 4 |
| AB117 | PBI 0 24hr | 2.1 | 60 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AB180 | PBI 0 24hr | 1.98 | 75 | fixed frozen | + | + | 0 | 0 | 20 | 7 |
| AB181 | PBI 0 24hr | 2.1 | 117 | fixed frozen | + | + | 0 | 0 | 20 | 6 |
| AB197 | PBI 0 24hr | 2.05 | 115 | fixed frozen | + | + | 0 | 0 | 20 | 6 |
| | | | | | | **Mean =** | 0 | 0 | 20 | 5.2 |
| | | | | | | | | | | |
| AB066 | PBI 0 72hr | 2.1 | 80 | fixed frozen | + | + | 0 | 0 | 20 | 6 |
| AB105 | PBI 0 72hr | 2.3 | 100 | fixed frozen | + | + | 1 | 0 | 20 | 5 |
| AB133 | PBI 0 72hr | 1.97 | 60 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AB191 | PBI 0 72hr | 1.9 | 95 | fixed frozen | + | + | 0 | 0 | 20 | 3 |
| AB194 | PBI 0 72hr | 2 | 65 | fresh frozen | + | + | 0 | 0 | 20 | 2 |
| | | | | | | **Mean =** | 0.2 | 0 | 20 | 3.8 |
| | | | | | | | | | | |
| | | | | | | **Mean for All PBI-0 Animals =** | 0.1 | 0.0 | 19.9 | 5.1 |

(continued ….)

## Table 4: Biosamples and neurobehavioral scores (Part 5 of 5)

| Sample ID | Expt Group | Total Amount of Plasma (ml) | Total Amount of CSF (µl) | brain | RBC in storage | MNC in storage | NSS | NIH | NBS | righting time (min) |
|---|---|---|---|---|---|---|---|---|---|---|
| AB079 | PBI III 15min | 1.85 | 0 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AB099 | PBI III 15min | 2.1 | 100 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AB148 | PBI III 15min | 2 | 0 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AB219 | PBI III 15min | 2 | 0 | fresh frozen | + | + | n/a | n/a | n/a | n/a |
| AB237 | PBI III 15min | 1.85 | 0 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| AB252 | PBI III 15min | 1.85 | 15 | fixed frozen | + | + | n/a | n/a | n/a | n/a |
| | | | | | | | | | | |
| AB074 | PBI III 60min | 2 | 0 | fixed frozen | + | + | n/a | n/a | n/a | 19 |
| AB121 | PBI III 60min | 2.1 | 0 | fixed frozen | + | + | n/a | n/a | n/a | 10 |
| AB207 | PBI III 60min | 1.97 | 0 | fixed frozen | + | + | n/a | n/a | n/a | 9 |
| AB220 | PBI III 60min | 1.98 | 60 | fresh frozen | + | + | n/a | n/a | n/a | 5 |
| AB248 | PBI III 60min | 1.9 | 35 | fixed frozen | + | + | n/a | n/a | n/a | 12 |
| | | | | | **Mean =** | | n/a | n/a | n/a | 11 |
| | | | **p-value (T test PBI-0 vs PBI-III at 1 hour) =** | | | | #DIV/0! | #DIV/0! | #DIV/0! | 0.149 |
| | | | | | | | | | | |
| AB120* | PBI III 6hr | 1.8 | 64 | fixed frozen | + | + | 6 | 0 | 11 | 17 |
| AB147 | PBI III 6hr | 1.9 | 60 | fixed frozen | + | + | 10 | 2 | 8 | 35 |
| AB201 | PBI III 6hr | 1.95 | 0 | fixed frozen | + | + | 10 | 2 | 8 | 17 |
| AB218 | PBI III 6hr | 1.85 | 70 | fresh frozen | + | + | 13 | 2 | 8 | * |
| AB236 | PBI III 6hr | 2 | 15 | fixed frozen | + | + | 4 | 0 | 16 | 7 |
| AB251 | PBI III 6hr | 1.9 | 30 | fixed frozen | + | + | 8 | 2 | 12 | 6 |
| AB268 | PBI III 6hr | 2 | 0 | fresh frozen | + | + | 9 | 1 | 10 | 36 |
| | | | | | **Mean =** | | 8.6 | 1.3 | 10.4 | 19.7 |
| | | | **p-value (T test PBI-0 vs PBI-III at 6 hours) =** | | | | 7.47E-05 | 0.014 | 5.47E-05 | 0.039 |
| | | | | | | | | | | |
| AB068 | PBI III 24hr | 1.85 | 0 | fixed frozen | + | + | 7 | 2 | 13 | 30 |
| AB138 | PBI III 24hr | 2 | 0 | fixed frozen | + | + | 6 | 0 | 16 | 9 |
| AB246 | PBI III 24hr | 1.95 | 0 | fixed frozen | + | + | 10 | 2 | 13 | 6 |
| AB247 | PBI III 24hr | 1.87 | 10 | fixed frozen | + | + | 10 | 2 | 12 | 8 |
| AB265 | PBI III 24hr | 1.95 | 70 | fixed frozen | + | + | 7 | 1 | 15 | 21 |
| AB266 | PBI III 24hr | 1.95 | 0 | fresh frozen | + | + | 7 | 0 | 16 | 9 |
| | | | | | **Mean =** | | 7.8 | 1.2 | 14.2 | 13.8 |
| | | | **p-value (T test PBI-0 vs PBI-III at 24 hours) =** | | | | 3.36E-06 | 0.027 | 3.68E-05 | 0.079 |
| | | | | | | | | | | |
| AB067* | PBI III 72hr | 2.2 | 0 | fixed frozen | + | + | 12 | 0 | 11 | 17 |
| AB106 | PBI III 72hr | 2.15 | 100 | fixed frozen | + | + | 5 | 0 | 16 | 33 |
| AB156 | PBI III 72hr | 2.2 | 30 | fixed frozen | + | + | 4 | 0 | 18 | 11 |
| AB160 | PBI III 72hr | 2.17 | 60 | fixed frozen | + | + | 3 | 0 | 18 | 7 |
| AB195* | PBI III 72hr | 2.05 | 0 | fresh frozen | + | + | 5 | 0 | 18 | 7 |
| AB227 | PBI III 72hr | 2 | 90 | fixed frozen | + | + | 7 | 0 | 12 | 14 |
| | | | | | **Mean =** | | 6 | 0 | 15.5 | 14.8 |
| | | | **p-value (T test PBI-0 vs PBI-III at 72 hours) =** | | | | 0.003 | #DIV/0! | 0.013 | 0.035 |
| | | | | | | | | | | |
| | | | **Mean for All PBI-III Animals =** | | | | 7.5 | 0.8 | 13.2 | 15 |
| | | | **p-value (T test All PBI-0 vs All PBI-III) =** | | | | 1.19E-11 | 0.002 | 1.67E-08 | 7.96E-05 |

## Table 5a:  List of FPI-III 15 Min proteins (p$\leq$ 10-3)

| Protein | Accession Number | p value | Controls | FPI 15m | FPI 1h | FPI 6h | FPI 24h | FPI 72h | PBI 15m | PBI 1h | PBI 6h | PBI 24h | PBI 72h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Voltage-dependent P/Q-type calcium channel alpha-1A subunit* | Q2YS37 | **4.315E-11** | **0** | **0.5** | **0** | **0** | **0** | **0** | **0.25** | **0** | **0** | **0** | **0** |
| Na+ channel (Fragment) | Q63360 | 9.039E-07 | 0.015625 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Putative integral membrane transport protein | O70609 | 2.158E-05 | 0.078125 | 0.75 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.75 | 0 |
| Probable saccharopine dehydrogenase (EC 1.5.1.9) | Q6AY30 | 3.929E-05 | 0.265625 | 1.5 | 0.5 | 0.25 | 0.25 | 0.5 | 0 | 0.25 | 0.5 | 0 | 0 |
| *Fucosyltransferase 11* | Q68FV3 | 4.97E-05 | 0.140625 | 1 | 0 | 0 | 0 | 0 | 1 | 0.5 | 0.25 | 0 | 0 |
| *UDP-glucuronosyltransferase 1-3* | Q64637 | 5.538E-05 | 0.03125 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Feline leukemia virus subgroup C receptor-related protein 2* | P60815 | **5.538E-05** | **0.03125** | **0.5** | **0.25** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| Putative taste receptor T2R20 (T2R6) | Q67ET0 | 5.538E-05 | 0.03125 | 0.5 | 0.5 | 0.5 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0 |
| Putative taste receptor T2R14 | Q67ET6 | 6.85E-05 | 0.0625 | 0.75 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0.25 | 0.25 | 0 |
| ORF protein | Q62693 | 9.437E-05 | 0.09375 | 0.75 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 |
| *Orphan nuclear receptor HZF-2* | Q62702 | **0.00022249** | **0.078125** | **0.75** | **0** | **0** | **0** | **0** | **0** | **0.25** | **0.5** | **0** | **0** |
| Tcrb protein | Q5BJL9 | 0.0002249 | 0.078125 | 0.75 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0.25 |
| *Vomeronasal V1r-type receptor V1rg11* | Q5J3G0 | **0.00022259** | **0.203125** | **1** | **0.25** | **0.25** | **0.5** | **0.25** | **0.25** | **0** | **0** | **0** | **0.5** |
| *Acetyl-Coenzyme A carboxylase 2* | Q1HEC0 | 0.0003921 | 0.1875 | 1 | 0.75 | 0 | 0.25 | 0 | 0.5 | 0.25 | 0.25 | 0.25 | 0.25 |
| *Beta-1,3-N-acetylglucosaminyltransferase radical fringe* | Q9R1U9 | **0.0005106** | **0.140625** | **1** | **0** | **0** | **0** | **0** | **0** | **1** | **0.25** | **0.25** | **0** |
| Progestin membrane receptor alpha | Q4PU88 | 0.0005437 | 0.046875 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.5 |
| **Plasmolipin (Plasma membrane proteolipid)** | P47987 | **0.0005437** | **0.046875** | **0.5** | **0** | **0** | **0** | **0** | **0.25** | **0** | **0.25** | **0.5** | **0** |
| LRRGT00159 | Q6QI49 | 0.0005437 | 0.046875 | 0.5 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.25 | 0 | 0 |
| Carbohydrate kinase-like | Q3MID4 | 0.0005437 | 0.046875 | 0.5 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0 | 0.25 |
| Vascular chymase | O70500 | 0.0005437 | 0.046875 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 |
| NADP-dependent leukotriene B4 12-hydroxydehydrogenase | P97584 | 0.000581 | 0.09375 | 0.75 | 0.25 | 0.25 | 0 | 0 | 0.25 | 0.25 | 0.25 | 0 | 0 |
| GDP-mannose pyrophosphorylase A | Q5XIC1 | 0.0005998 | 0.171875 | 1 | 0.25 | 0.25 | 0 | 0 | 0 | 0.75 | 0.5 | 0.75 | 0.25 |
| *Neural F box protein NFB42* | Q9Z1X8 | **0.0007416** | **0.125** | **0.75** | **0** | **0.25** | **0** | **0** | **0** | **0** | **0.25** | **0.25** | **0** |
| Mannosidase 2, alpha B1 | Q6P762 | 0.0007616 | 0.296875 | 1.25 | 0.5 | 0 | 0.25 | 0 | 0.25 | 0.25 | 1 | 0.75 | 1 |
| *Succinyl-CoA ligase [GDP-forming] alpha-chain, mitochondrial* | P13086 | 0.0008381 | 0.15625 | 1 | 0.25 | 0.25 | 0.5 | 0 | 0.5 | 0.25 | 0 | 0.25 | 0 |

# Table 5b: List of FPI-III 1 Hour proteins (p≤ 10-3)

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| *Ischemia related factor vof-16* | **Q8K4N0** | **4.315E-11** | **0** | **0.25** | **0.5** | **0** | **0** | **0** | **0** | **0** | **0.25** | **0** | **0** |
| *GTP cyclohydrolase 1* | **P22288** | **2.331E-09** | **0.03125** | **0.25** | **0.75** | **0.25** | **0** | **0** | **0** | **0** | **0.25** | **0** | **0** |
| *Cytochrome c oxidase polypeptide VIc-2* | P11951 | 9.039E-07 | 0.015625 | 0.25 | 0.5 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0 |
| **CL3BB** | **Q4LDM5** | **9.039E-07** | **0.015625** | **0.25** | **0.5** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| *Hypothetical protein LOC312102* | Q5BK23 | 2.979E-06 | 0.0625 | 0 | 0.75 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 |
| *40S ribosomal protein S10* | **P63326** | **5.538E-05** | **0.03125** | **0** | **0.5** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| *3-mercaptopyruvate sulfurtransferase (MST)* | **P97532** | **5.538E-05** | **0.03125** | **0** | **0.5** | **0** | **0** | **0** | **0** | **0.25** | **0** | **0** | **0** |
| *Rps2rl* | O35805 | 5.538E-05 | 0.03125 | 0 | 0.5 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0 |
| *Vomeronasal V1r-type receptor V1re20* | **Q5J3F4** | **0.0001091** | **0.15625** | **0** | **1** | **0** | **0** | **0** | **0.25** | **0.25** | **0.5** | **0** | **0.75** |
| *Junction adhesion molecule 2* | Q3MHC0 | 0.0005437 | 0.046875 | 0 | 0.5 | 0 | 0 | 0.5 | 0 | 0 | 0.5 | 0 | 0 |
| *Cell surface glycoprotein gp42 precursor* | P23505 | 0.0005437 | 0.046875 | 0 | 0.5 | 0 | 0.25 | 0 | 0.25 | 0 | 0.75 | 0 | 0 |
| *Ly49 stimulatory receptor 5* | Q5DLU5 | 0.0005437 | 0.046875 | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.25 |
| *Glia maturation factor beta (GMF-beta)* | **Q63228** | **0.0005437** | **0.046875** | **0** | **0.5** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** |
| **Kinesin heavy chain isoform 5C (Kinesin heavy chain neuron-specific 2)** | **P56536** | **0.0005437** | **0.046875** | **0.25** | **0.5** | **0** | **0** | **0** | **0** | **0** | **0** | **0.25** | **0** |
| **40S ribosomal protein S7 (S8)** | **P62083** | **0.0005437** | **0.046875** | **0.25** | **0.5** | **0** | **0** | **0** | **0** | **0.25** | **0** | **0** | **0.25** |
| *G protein-coupled receptor kinase* | P97548 | 0.0005437 | 0.046875 | 0.25 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.25 |
| *Actin-like protein 2 (Actin-related protein 2)* | Q5M7U6 | 0.000581 | 0.09375 | 0 | 0.75 | 0 | 0 | 0.25 | 0 | 0.25 | 0.25 | 0.25 | 0 |
| *Potassium channel modulatory factor 1 (Predicted)* | Q5XIQ2 | 0.000581 | 0.09375 | 0 | 0.75 | 0 | 0 | 0.25 | 0.25 | 0.25 | 0.75 | 0.5 | 0.25 |

## Table 5c:  List of FPI-III 6 Hour proteins (p≤ 10-3)

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| Cdig2 protein (Fragment) | Q642F9 | 4.315E-11 | 0 | 0 | 0 | 0.5 | 0 | 0.25 | 0 | 0 | 0 | 0.25 | 0 |
| Putative taste receptor T2R20 (T2R16) | Q67ET0 | 5.538E-05 | 0.03125 | 0.5 | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 |
| Skeletal troponin C | Q304F3 | 5.538E-05 | 0.03125 | 0 | 0 | 0.5 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| Psmd8 protein (Fragment) | Q3B8P5 | 5.538E-05 | 0.03125 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0.25 | 0.25 | 0 | 0 |
| Hypothetical protein | Q569A8 | 5.538E-05 | 0.03125 | 0 | 0 | 0.5 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0.25 |
| Neurexin-1-beta precursor (Neurexin 1-beta) | Q63373 | 6.85E-05 | 0.0625 | 0 | 0.25 | 0.75 | 0 | 0.25 | 0.75 | 0.25 | 0.5 | 0.25 | 0 |
| Phosducin-like 3 | Q4KLJ8 | 0.0005437 | 0.046875 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 |

## Table 5d:  List of FPI-III 24 Hour proteins (p≤ 10-3)

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| Testicular cell adhesion molecule 1 (TCAM1) | Q9Z133 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0.5 | 0 | 0.75 | 0 | 0.25 | 0.75 | 0 |
| T-cell ecto-ADP-ribosyltransferase 2 precursor | P20974 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0.25 | 0 | 0 | 0 |
| **Neuromedin U receptor 2 (NMU-R2)** | **Q9ESQ4** | **5.538E-05** | **0.03125** | **0** | **0** | **0** | **0.5** | **0** | **0** | **0** | **0.25** | **0** | **0** |
| Rat bone protein I (BP-I) (Fragment) | Q63069 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 | 0.25 | 0 |
| ARF-related protein (ARP) | Q63055 | 5.538E-05 | 0.03125 | 0.25 | 0.25 | 0.25 | 0.5 | 0.25 | 0.25 | 0.25 | 0 | 0 | 0 |
| **Synaptonemal complex protein 3 (SCP-3)** | **Q63520** | **5.538E-05** | **0.03125** | **0.25** | **0.25** | **0.25** | **0.5** | **0.25** | **0.5** | **0** | **0** | **0.25** | **0** |
| 60 kDa lysophospholipase | O88202 | 5.538E-05 | 0.03125 | 0.25 | 0.25 | 0 | 0.5 | 0.25 | 0.25 | 0 | 0.25 | 0.25 | 0 |
| Hypothetical protein RGD1308517 | Q3B7T8 | 0.00497 | 0.21875 | 0.25 | 0.25 | 0.5 | 1.25 | 0.25 | 0.25 | 0.5 | 0.25 | 0.25 | 0.25 |
| Testicular ecto-ATPase | Q5RJP4 | 0.0005437 | 0.046875 | 0 | 0 | 0 | 0.5 | 0 | 0.25 | 0 | 0 | 0 | 0 |
| Glutathione peroxidase 2 | P83645 | 0.0005437 | 0.046875 | 0 | 0 | 0 | 0.5 | 0.25 | 0.25 | 0 | 0 | 0 | 0.5 |
| Protease, serine, 16 (Thymus) | Q3MHS0 | 0.0005437 | 0.046875 | 0.25 | 0.25 | 0 | 0.5 | 0 | 0.25 | 0 | 0 | 0 | 0.5 |
| Hypothetical protein LOC619440 | Q5XI97 | 0.000581 | 0.09375 | 0 | 0.25 | 0.25 | 0.75 | 0.25 | 0 | 0 | 0.5 | 0.5 | 0 |
| Protein AATF (Apoptosis-antagonizing transcription factor) | Q9QYW0 | 0.000581 | 0.09375 | 0 | 0 | 0 | 0.75 | 0.25 | 0.5 | 1 | 1 | 0.75 | 0 |
| Rab proteins geranylgeranyltransferase component A 1 | P37727 | 0.0006956 | 0.234375 | 0 | 0.5 | 0 | 1.25 | 0.25 | 0 | 0 | 0.75 | 0.25 | 0.5 |

**Table 5e:  List of FPI-III 72 Hour proteins (p≤ 10-3)**

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| *Syntaxin-1A (Neuron-specific antigen HPC-1)* | **P32851** | **2.331E-09** | **0.03125** | 0.25 | 0 | 0 | 0 | 0.75 | 0 | 0 | 0.25 | 0 | 0 |
| *Nucleic acid binding protein* | Q4JFV5 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 | 0 |
| *Muellerian-inhibiting factor precursor (MIS)* | P49000 | 5.432E-06 | 0.140625 | 0 | 0.5 | 0.25 | 0 | 1.25 | 1 | 0 | 0.5 | 0.25 | 0 |
| *Butyrophilin-like protein 2 precursor* | Q6MG97 | 2.158E-05 | 0.078125 | 0 | 0.25 | 0 | 0.75 | 0.75 | 0 | 0.25 | 0 | 0 | 0.25 |
| *Vomeronasal V1r-type receptor V1rc39* | Q5I3I1 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0.75 | 0 | 0 | 0 | 0 | 0 |
| *Hypothetical protein (Fragment)* | Q6GX83 | 5.538E-05 | 0.03125 | 0 | 0.25 | 0 | 0 | 0.5 | 0.25 | 0 | 0.25 | 0 | 0 |
| **Bms1l protein [Fragment]** | **Q561Z9** | **5.538E-05** | **0.03125** | 0 | 0 | 0 | 0.25 | 0.5 | 0.25 | 0 | 0.25 | 0.25 | 0 |
| *Receptor-type tyrosine-protein phosphatase alpha* | Q03348 | 5.538E-05 | 0.03125 | 0 | 0.25 | 0 | 0 | 0.5 | 0 | 0 | 0.25 | 0 | 0.25 |
| *Similar to cDNA sequence BC021608* | Q66H45 | 6.85E-05 | 0.0625 | 0 | 0 | 0 | 0.25 | 0.75 | 0.25 | 0 | 0.25 | 0 | 0 |
| *Wilms' tumor protein homolog* | P49952 | 0.0003496 | 0.15625 | 0.25 | 0.5 | 0 | 0 | 1 | 0.25 | 0 | 0.5 | 0 | 0.25 |
| *Junction adhesion molecule 2* | Q3MHC0 | 0.0005437 | 0.046875 | 0 | 0.5 | 0 | 0 | 0.5 | 0 | 0 | 0.5 | 0 | 0 |
| *Ac2-086* | Q7TPK4 | 0.0005437 | 0.046875 | 0 | 0 | 0 | 0 | 0.5 | 0.25 | 0 | 0.25 | 0 | 0.25 |
| *Cysteine-rich secretory protein 1 precursor* | P12020 | 0.0005437 | 0.046875 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0.25 | 0.5 | 0.25 |

# Table 6a:  List of PBI-III 15 Min proteins (p≤ 10-4)  Part 1 of 3

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| Trace amine-associated receptor 8a | Q923X9 | 4.315E-11 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 |
| PDZ domain-containing protein 1 | Q9JI40 | 1.265E-09 | 0.21875 | 0 | 0.25 | 0 | 0.25 | 0 | 1.75 | 0 | 0.25 | 0.5 | 0.5 |
| Testicular cell adhesion molecule 1 | Q9Z133 | 2.331E-09 | 0.03125 | 0 | 0 | 0 | 0.5 | 0 | 0.75 | 1 | 0.25 | 0.75 | 0 |
| Serine hydroxymethyl transferase 2 (Mitochondrial) | Q5U3Z7 | 2.49E-09 | 0.15625 | 0.25 | 0.25 | 0 | 0.25 | 0.5 | 1.5 | 0 | 0.5 | 0.5 | 0 |
| Thyrotropin-releasing hormone receptor | Q01717 | 3.444E-09 | 0.046875 | 0.25 | 0 | 0 | 0 | 0.25 | 1 | 0 | 0.25 | 0 | 0 |
| ATP-sensitive inward rectifier potassium channel 1 | P35560 | 4.172E-09 | 0.0078125 | 0 | 0 | 0 | 0 | 0.25 | 1 | 0 | 0.25 | 0.25 | 0.25 |
| 2'-5' oligoadenylate synthetase 2-like protein | Q5MYT9 | 2.101E-08 | 0.125 | 0.25 | 0.5 | 0.25 | 0 | 0 | 1.25 | 0 | 0.5 | 0.5 | 0 |
| Cathepsin L precursor | P07154 | 1.511E-07 | 0.328125 | 0.25 | 0.25 | 0.25 | 0 | 0.25 | 2 | 0.25 | 1 | 1 | 1 |
| Low mol. weight phosphotyrosine protein phosphatase | P41498 | 1.791E-07 | 0.046875 | 0 | 0.25 | 0 | 0 | 0 | 0.75 | 0.5 | 0 | 0 | 0 |
| Branched-chain-amino-acid aminotransferase, mitochondrial | O35854 | 2.63E-07 | 0.046875 | 0 | 0 | 0 | 0 | 0 | 1.25 | 0 | 0.25 | 0 | 0 |
| Tubulin tyrosine ligase-like 1 (Predicted) | Q5PPI9 | 3.294E-07 | 0.125 | 0 | 0.25 | 0 | 0 | 0 | 1.25 | 0.25 | 0.25 | 0.25 | 0.5 |
| Carnitine deficiency-associated protein expressed in ventricle 1 | P83829 | 3.595E-07 | 0.28125 | 0.5 | 0.25 | 0 | 0.25 | 0.25 | 2 | 0.5 | 1.25 | 0.25 | 0.5 |
| Ribonuclease 12 | Q5GAL8 | 3.659E-07 | 0.078125 | 0 | 0 | 0 | 0 | 0.25 | 1 | 0 | 0.25 | 0.25 | 0 |
| Caspase recruitment domain-containing protein 9 (rCARD9) | Q9EPY0 | 4.56E-07 | 0.484375 | 0.25 | 0.5 | 0 | 0 | 0.5 | 2.25 | 0 | 1.25 | 0.5 | 0.75 |
| Hypothetical protein LOC361016 | Q4V7B1 | 4.584E-07 | 0.234375 | 0.25 | 0.25 | 0 | 0.25 | 0.25 | 1.75 | 0 | 0 | 0.5 | 0.5 |
| PH domain leucine-rich repeat protein phosphatase | Q9WTR8 | 5.646E-07 | 0.65625 | 0.5 | 0.75 | 0.75 | 0.25 | 0.25 | 3.5 | 0.75 | 1.75 | 0.75 | 1.25 |
| Cd5 protein | Q4KLZ9 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.25 | 0 | 0 |
| Similar to RIKEN cDNA 2810037C03 | Q499W2 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.5 | 0 | 0 |
| Axl receptor tyrosine kinase | O55059 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0 | 0 | 0 | 0 |
| Myosin light polypeptide 6 | Q64119 | 9.039E-07 | 0.015625 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0.25 | 0 | 0 | 0 |
| Olfactory receptor 1 | P70526 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 |
| Src family associated phosphoprotein 1 | Q4V7G1 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.25 | 0 | 0 |
| Putative pheromone receptor V2R2a (Fragment) | Q99PC1 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 |
| BRCA1-associated RING domain protein 1 beta isoform | Q6J724 | 9.039E-07 | 0.015625 | 0.25 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 |
| Placental prolactin-like protein K precursor | Q9JKL9 | 9.039E-07 | 0.015625 | 0.25 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0 | 0 | 0 | 0.25 |
| Drebrin-like protein | Q9JHL4 | 9.746E-07 | 0.140625 | 0 | 0 | 0.25 | 0.5 | 0.25 | 1.25 | 0.75 | 0.5 | 0.25 | 0.25 |
| Hypothetical protein RGD1305287 | Q5I0F1 | 1.391E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0.25 | 1 | 0 | 0.25 | 0.25 | 0 |
| Beta-1,3-N-acetylglucosaminyltransferase lunatic fringe | Q924T4 | 1.737E-06 | 0.53125 | 0.25 | 0.25 | 0.25 | 0 | 0.5 | 2.5 | 0.75 | 2.5 | 0.75 | 0.25 |
| Hypothetical protein RGD1307084 | Q5I0I4 | 1.967E-06 | 0.28125 | 0.25 | 0.25 | 0 | 0.5 | 0 | 1.75 | 0 | 0.25 | 0.25 | 0 |
| Neurexophilin-4 precursor | Q9Z2N4 | 2.33E-06 | 0.125 | 0 | 0 | 0 | 0.25 | 0 | 1.25 | 0.5 | 0.75 | 0 | 0.25 |

## Table 6a: List of PBI-III 15 Min proteins (p≤ 10-4) Part 2 of 3

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| *Killer cell lectin-like receptor subfamily H, member 1* | Q8K4F1 | 2.503E-06 | 0.15625 | 0 | 0 | 0 | 0 | 0 | 1.25 | 0 | 0.25 | 0.5 | 0.25 |
| *Charged multivesicular body protein 5* | Q4QQV8 | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0 | 0.25 | 0 | 0 |
| *Fln29 protein* | Q5M806 | 6.35E-06 | 0.328125 | 0 | 0 | 0.5 | 0.25 | 0 | 1.75 | 0.75 | 1 | 0.25 | 0 |
| *Gonadotropin-regulated long chain acyl-CoA synthetase* | Q924N5 | 7.544E-06 | 0.390625 | 0.25 | 0 | 0 | 0.5 | 0 | 2 | 0.25 | 0.25 | 0.25 | 0 |
| ***Calcium/calmodulin-dependent protein kinase type II alpha chain*** | **P11275** | **1.193E-05** | **0.1875** | **0** | **0** | **0** | **0** | **0** | **1.25** | **0.25** | **0.5** | **0** | **0.25** |
| *Gut-enriched kruppel-like factor (Kruppel-like factor 4) (Gut)* | Q923V7 | 1.446E-05 | 0.8125 | 0.25 | 0.75 | 1.5 | 0.25 | 1.25 | 2.75 | 1 | 1.5 | 0.5 | 1 |
| *Ab2-060* | Q7TP64 | 1.473E-05 | 0.046875 | 0 | 0 | 0 | 0 | 0.25 | 0.75 | 0.25 | 0.25 | 0.25 | 0 |
| *Follistatin-related protein 3* | Q99PW7 | 1.473E-05 | 0.046875 | 0 | 0.5 | 0.25 | 0 | 0 | 0.75 | 0.25 | 0 | 0 | 0.25 |
| *GA repeat binding protein, beta 1* | Q4KM28 | 1.473E-05 | 0.046875 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0 | 0 | 0 | 0 |
| *Epididymis-specific RNase-like (Ribonuclease 9)* | Q5QJV4 | 1.473E-05 | 0.046875 | 0.5 | 0 | 0 | 0 | 0 | 0.75 | 0.25 | 0.25 | 0 | 0 |
| *Hypothetical protein RGD1307598* | Q32PZ9 | 1.946E-05 | 0.84375 | 1.25 | 1 | 0.75 | 1 | 0.5 | 2.25 | 0.75 | 1 | 0.5 | 1 |
| *TNFAIP3 interacting protein 2* | Q4V7E7 | 1.987E-05 | 0.125 | 0 | 0.25 | 0.75 | 1 | 0.5 | 1 | 0 | 0.5 | 0.25 | 0.25 |
| *Aa1114* | Q7TQI2 | 2.007E-05 | 0.140625 | 0 | 0.25 | 0.25 | 0.25 | 0.25 | 1.25 | 0 | 0.75 | 0.75 | 0 |
| ***Thyroliberin*** | **P01150** | **2.158E-05** | **0.078125** | **0.5** | **0.25** | **0** | **0** | **0.25** | **0.75** | **0** | **0.5** | **0.25** | **0** |
| *Suppressor of cytokine signaling 2 (SOCS-2)* | O88582 | 2.601E-05 | 0.21875 | 0.25 | 0.25 | 0.25 | 0 | 0.25 | 1.5 | 0.5 | 0.5 | 0.25 | 0.5 |
| *Inhibin beta C chain precursor (Activin beta-C chain)* | Q9WUK5 | 2.627E-05 | 0.25 | 0.5 | 0 | 1 | 0.25 | 0.25 | 1.5 | 0 | 0.5 | 0.25 | 0.25 |
| *Hypothetical protein LOC300963* | Q4KLY0 | 3.237E-05 | 0.453125 | 0.75 | 0.5 | 0.25 | 0.5 | 0.75 | 2 | 0.75 | 0.75 | 0.25 | 0.75 |
| *Nuclear receptor coactivator p120* | Q5TLG7 | 4.133E-05 | 0.21875 | 0.25 | 0.75 | 0.25 | 0.5 | 0.25 | 1.25 | 0.25 | 0.75 | 0.25 | 0.5 |
| *Estradiol 17-beta-dehydrogenase 1* | P51657 | 4.133E-05 | 0.21875 | 0 | 0.25 | 0 | 0.25 | 0 | 1.25 | 0 | 0.25 | 0 | 0 |
| *Hypothetical protein LOC361399* | Q3ZAV8 | 4.481E-05 | 0.4375 | 0.25 | 0.25 | 0.75 | 0.25 | 0.5 | 2 | 0.75 | 1.25 | 0.75 | 0.25 |
| *Beta-2-glycoprotein 1 (Apolipoprotein H)* | P26644 | 4.512E-05 | 7.53125 | 9.5 | 6.5 | 8.75 | 7.5 | 6.5 | 12 | 8.25 | 8.75 | 7.5 | 9 |
| *Zinc finger CCCH type domain containing 8* | Q6AYB0 | 4.73E-05 | 0.53125 | 0.25 | 0.25 | 0.25 | 0.75 | 0.5 | 2.25 | 0.5 | 1.25 | 0.5 | 1.5 |
| *Hypothetical protein RGD1307787* | Q5BK61 | 4.922E-05 | 0.40625 | 0.5 | 1 | 0.5 | 0.5 | 1.25 | 2 | 0 | 0.75 | 0.25 | 0.25 |
| *Muellerian-inhibiting factor* | P49000 | 4.97E-05 | 0.140625 | 0 | 0.5 | 0.25 | 0 | 1.25 | 1 | 0 | 0.5 | 0.25 | 0 |
| *Fucosyltransferase 11* | Q68FV3 | 4.97E-05 | 0.140625 | 1 | 0 | 0 | 0 | 0.25 | 1 | 0.5 | 0.5 | 0 | 0 |
| ***Hypothetical protein RGD1309552*** | **Q566C8** | **4.97E-05** | **0.140625** | **0.25** | **0.25** | **0** | **0** | **0.25** | **1** | **0.25** | **0.5** | **0** | **0.25** |
| *Synaptonemal complex protein 3 (SCP-3)* | Q63520 | 5.538E-05 | 0.03125 | 0.25 | 0.25 | 0.25 | 0.5 | 0.25 | 0.5 | 0 | 0 | 0.25 | 0 |
| *Leucine-rich repeat-containing protein 59 (Protein p34)* | Q5RJR8 | 5.538E-05 | 0.03125 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 |
| *Hypothetical protein* | Q498D3 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0 |

**Table 6a: List of PBI-III 15 Min proteins (p≤ 10-4) Part 3 of 3**

| Protein | Accession Number | P value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| Tdrd7 protein | Q5FVD0 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 0 | 0 | 0 | 0 |
| RAB30, member RAS oncogene family (Predicted) | Q5BK72 | 5.538E-05 | 0.03125 | 0 | 0.5 | 0.25 | 0 | 0.25 | 0.5 | 0 | 0.25 | 0 | 0 |
| Ab2-379 | Q7TP46 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 0.5 | 0 | 0.25 | 0 |
| HES-related repressor protein 2 HERP2 (Fragment) | Q8VIH4 | 5.538E-05 | 0.03125 | 0 | 0 | 0.25 | 0 | 0 | 0.5 | 0.25 | 0 | 0.5 | 0 |
| Hypothetical protein LOC498176 | Q5U3Z5 | 5.538E-05 | 0.03125 | 0.25 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.25 | 0.25 | 0 |
| Ectodermal-neural cortex 1 | Q2V9T0 | 5.622E-05 | 0.40625 | 0.25 | 0.75 | 0 | 0.5 | 0 | 1.75 | 0.75 | 1.5 | 0.25 | 0.5 |
| Neurexin-1-beta precursor (Neurexin I-beta) | Q63373 | 6.85E-05 | 0.0625 | 0 | 0.25 | 0.75 | 0 | 0.25 | 0.75 | 0.25 | 0.5 | 0 | 0 |
| Disabled-1 | Q8CJH2 | 6.85E-05 | 0.0625 | 0 | 0.25 | 0.25 | 0 | 0 | 0.75 | 0 | 0.5 | 0 | 0 |
| Four and a half LIM domains protein 2 (FHL-2) | O35115 | 7.055E-05 | 1.140625 | 1.5 | 0.75 | 1 | 1 | 0.5 | 3 | 1.25 | 2.75 | 1.5 | 0.5 |
| Spnb4 protein (Fragment) | Q5BJU8 | 8.087E-05 | 0.296875 | 0.5 | 0.5 | 0.5 | 0.25 | 0.75 | 1.5 | 0.25 | 1.5 | 0.25 | 0.25 |
| 78 kDa glucose-regulated protein precursor (GRP 78) | P06761 | 8.285E-05 | 2.0625 | 1.75 | 1 | 0.5 | 2.5 | 0.75 | 5.5 | 1.75 | 4.25 | 2.75 | 3 |
| Glutathione s-transferase M5 (EC 2.5.1.18) | Q9Z1B2 | 9.437E-05 | 0.09375 | 0 | 0 | 0 | 0.5 | 0 | 0.75 | 0.25 | 0.25 | 0 | 0.5 |
| LRRGT00162 | Q6Q146 | 9.437E-05 | 0.125 | 0.25 | 0 | 0.25 | 0 | 0.25 | 1 | 0.25 | 0 | 0.25 | 0 |
| Plectin 1 | Q6S3A5 | 9.437E-05 | 0.09375 | 0 | 0 | 0.5 | 0.25 | 0 | 0.75 | 0 | 0.5 | 0.25 | 0 |
| Hypothetical protein | Q2MHH0 | 9.437E-05 | 0.125 | 0.75 | 0.5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0.5 |
| Mitochondrial 2-oxodicarboxylate carrier | Q99JD3 | 9.437E-05 | 0.09375 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0.5 | 0.5 | 0 | 0 |
| Tissue factor pathway inhibitor-2 precursor | Q8CF99 | 9.437E-05 | 0.09375 | 0 | 0 | 0 | 0.5 | 0 | 0.75 | 0.25 | 0.5 | 0 | 0.25 |
| Similar to Dner protein | Q5U215 | 9.437E-05 | 0.125 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0.5 |
| Serine/threonine/tyrosine interacting-like 1 | Q4G076 | 9.437E-05 | 0.09375 | 0.25 | 0 | 0 | 0 | 0 | 0.75 | 0 | 0 | 0 | 0 |
| Aminopeptidase O (AP-O) | P69527 | 9.548E-05 | 0.359375 | 1 | 0.25 | 0.25 | 0 | 0 | 2 | 0.25 | 1 | 0.25 | 0.75 |

# Table 6b:  List of PBI-III 1 Hour proteins (p≤ 10-4)

| Protein | Accession Number | p value | Controls | FPI 15m | FPI 1 h | FPI 6 h | FPI 24h | FPI 72 h | PBI 15m | PBI 1 h | PBI 6 h | PBI 24h | PBI 72 h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Tazarotene-induced protein 1 | Q58NB7 | 4.315E-11 | 0 | 0.25 | 0.25 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 |
| Pulmonary surfactant-associated protein C | P11685 | 7.961E-08 | 0.078125 | 0.25 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 |
| Olfactory receptor (Fragment) | Q9QWX1 | 1.791E-07 | 0.046875 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 |
| Sodium/calcium exchanger 1 splice variant NaCa10 | Q9Z0T8 | 9.039E-07 | 0.015625 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0.75 | 0 | 0 | 0 |
| 40S ribosomal protein S9 | P29314 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.25 | 0 | 0 |
| 50 kD glycoprotein (Rh50) | O88300 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.25 | 0 | 0 |
| Hypothetical protein LOC500909 | Q5M862 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.25 | 0 |
| Phosphoglycerate dehydrogenase like 1 | Q3KR85 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0.5 | 0 | 0 |
| **Nerve growth factor-regulated G-protein-coupled receptor 1** | **Q9JKM5** | **9.746E-07** | **0.140625** | **0** | **0** | **0.25** | **0** | **0** | **0** | **1.25** | **0** | **0** | **0.25** |
| Apoptosis-antagonizing transcription factor | Q9QYW0 | 1.820E-06 | 0.09375 | 0 | 0 | 0 | 0.75 | 0.25 | 0.5 | 1 | 1 | 0.75 | 0 |
| Aa2-296 | Q7TP76 | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0 | 0 | 0 |
| Putative taste receptor T2R33 | Q67ER8 | 2.979E-06 | 0.0625 | 0.5 | 0 | 0 | 0 | 0 | 0.25 | 0.75 | 0.25 | 0 | 0 |
| Dnaj-like protein | Q9QZW8 | 6.716E-06 | 0.109375 | 0 | 0.25 | 0 | 0 | 0 | 0 | 1 | 0.25 | 0.25 | 0.75 |
| Proteasome subunit beta type 9 | P28077 | 1.010E-05 | 0.125 | 0.5 | 0 | 0 | 0 | 0.25 | 0.5 | 1.25 | 0.25 | 0.75 | 0.25 |
| Glutamate--cysteine ligase regulatory subunit | P48508 | 1.473E-05 | 0.046875 | 0.5 | 0 | 0 | 0 | 0.25 | 0 | 0.75 | 0.75 | 0.5 | 0.25 |
| **Mago-nashi-like proliferation-associated protein** | **Q27W02** | **1.473E-05** | **0.046875** | **0** | **0** | **0** | **0** | **0** | **0.25** | **0.75** | **0.25** | **0** | **0.25** |
| **Mas-related G-protein coupled receptor member D** | **Q7TN41** | **2.158E-05** | **0.078125** | **0** | **0** | **0** | **0** | **0.5** | **0** | **0.75** | **0.25** | **0** | **0** |
| Ankyrin repeat domain 13 (Predicted) | Q5U313 | 2.158E-05 | 0.078125 | 0 | 0.5 | 0 | 0.5 | 0 | 0.5 | 0.75 | 0.75 | 0.25 | 0.5 |
| Serine protease inhibitor Kazal-type 6 precursor | Q6IE47 | 2.158E-05 | 0.078125 | 0.25 | 0 | 0 | 0 | 0 | 0.25 | 0.75 | 0.25 | 0 | 0 |
| Hypothetical protein RGD1311362 | Q5BJV9 | 2.158E-05 | 0.078125 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.75 | 0.25 | 0 | 0.25 |
| Ab2-379 | Q7TP46 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.5 | 0.25 | 0.25 | 0 |
| **Ras-related protein Rab-3C** | **P62824** | **5.538E-05** | **0.03125** | **0** | **0** | **0** | **0** | **0** | **0** | **0.5** | **0** | **0** | **0** |
| Annexin A6 (Annexin VI) | P48037 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.25 | 0 | 0 |
| Hypothetical protein | Q32PX1 | 5.538E-05 | 0.03125 | 0.25 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0.25 | 0 | 0 |
| Protein kinase C delta-bindig protein | Q9Z1H9 | 6.850E-05 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.75 | 0.5 | 0 | 0.25 |
| **Achaete-scute homolog 2** | **P19360** | **8.725E-05** | **0.359375** | **0.5** | **0.25** | **0** | **0.75** | **0** | **0.5** | **1.75** | **0** | **0** | **0.75** |
| Hypothetical protein RGD1307700 | Q5I0I5 | 9.437E-05 | 0.09375 | 0 | 0.5 | 0 | 0 | 0 | 0.5 | 0.75 | 0.25 | 0 | 0.25 |

# Table 6c: List of PBI-III 6 Hour proteins (p≤ 10-4) Part 1 of 6

| Protein | Accession Number | p value | Controls | FPI 15m | FPI 1h | FPI 6h | FPI 24h | FPI 72h | PBI 15m | PBI 1h | PBI 6h | PBI 24h | PBI 72h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Prostaglandin G/H synthase 1 | PGHL_RAT | 1.369E-14 | 0.125 | 0 | 0.25 | 0 | 0.25 | 0.5 | 0 | 0.25 | 2 | 0 | 0.5 |
| WD repeat domain 78 | Q4V8G4 | 4.681E-13 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 1 | 0 | 0 |
| Proteasome subunit alpha type 4 | PSA4_RAT | 1.279E-12 | 0.09375 | 0 | 0 | 0.25 | 0 | 0 | 0.25 | 0 | 1.5 | 0 | 0.5 |
| **Beta-neoendorphin-dynorphin** | **PDYN_RAT** | **4.315E-11** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0** | **0.5** | **0** | **0** |
| Olfactory receptor | O35184 | 4.315E-11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.5 | 0 | 0 |
| Rat Ly6-C antigen | Q63318 | 4.315E-11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.5 | 0 | 0 |
| LOC502872 protein | Q4KM64 | 4.315E-11 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Protein FAM26B | FA26B_RAT | 9.286E-11 | 0.03125 | 0 | 0.25 | 0 | 0 | 0 | 0.25 | 0 | 1 | 0.25 | 0 |
| *Neuronal pentraxin-1* | **NPTX1_RAT** | **1.372E-10** | **0.1875** | **0.25** | **0.5** | **0** | **0.25** | **0.5** | **1** | **0.25** | **1.75** | **0.5** | **0.25** |
| Glucocorticoid receptor (GR) | GCR_RAT | 1.663E-09 | 0.828125 | 0.75 | 0.5 | 0 | 0.75 | 0.5 | 1.5 | 0.75 | 4 | 1.75 | 0.5 |
| CRAMP (Fragment) | Q71KM5 | 2.331E-09 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.75 | 0 | 0 |
| *Sdccag1 protein (Fragment)* | **Q5D022** | **2.943E-09** | **0.125** | **0.25** | **0.25** | **0.25** | **0.25** | **0** | **0.25** | **0.25** | **1.5** | **0.25** | **0** |
| Skeletal muscle and kidney enriched inositol phosphatase | Q5XIU8 | 3.444E-09 | 0.046875 | 0 | 0 | 0.25 | 0 | 0 | 0.25 | 0.25 | 1 | 0 | 0.25 |
| RGD1306356 protein | Q4G035 | 4.172E-09 | 0.078125 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 1 | 0.5 | 0 |
| *Neuromedin-B receptor (NMB-R)* | **NMBR_RAT** | **8.823E-09** | **0.171875** | **0** | **0.25** | **0** | **0** | **0** | **0** | **0** | **1.5** | **0** | **0.5** |
| NUAK family, SNF1-like kinase, 2 | Q66HE5 | 1.267E-08 | 0.671875 | 0.5 | 0.25 | 0.25 | 0.25 | 0.25 | 0.75 | 1 | 3.5 | 0.25 | 0.5 |
| Insulin responsive sequence DNA binding protein-1 | Q5ZQU0 | 1.3E-08 | 0.34375 | 0.5 | 0 | 0.25 | 0.25 | 0.75 | 1 | 0.5 | 2.5 | 0 | 0 |
| Myosin-9 (Myosin heavy chain, nonmuscle IIa) | MYH9_RAT | 1.412E-08 | 0.40625 | 0 | 0.25 | 0 | 0 | 0.25 | 0.5 | 0.5 | 2.25 | 0.25 | 0.75 |
| LOC290150 protein | Q562C1 | 2.104E-08 | 0.09375 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 0 | 1.25 | 0 | 0 |
| *Kinesin heavy chain (Ubiquitous kinesin heavy chain) (UKHC)* | **KINH_RAT** | **4.34E-08** | **0.78125** | **0.25** | **0** | **0.5** | **0.25** | **1** | **1** | **0.75** | **3.5** | **1.75** | **0.5** |
| LOC500180 protein | Q5M838 | 4.694E-08 | 0.3125 | 0.5 | 0 | 0 | 0.25 | 0.25 | 0.5 | 0.75 | 2.25 | 0.25 | 0.25 |
| Aldose reductase-like protein | Q91W30 | 4.835E-08 | 0.0625 | 0 | 0 | 0 | 0 | 0.25 | 1 | 0 | 1 | 0.25 | 0 |
| Fatty acid-binding protein, epidermal (E-FABP) | FABPE_RAT | 4.835E-08 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 1 | 0.5 | 0.25 |
| Type-II keratin Kb20 (Type-II keratin Kb2) | Q6IMF1 | 5.115E-08 | 0.640625 | 0.75 | 0.25 | 0.25 | 0.5 | 0.25 | 0.25 | 0.75 | 3.5 | 1.75 | 1 |
| **Heterogeneous nuclear ribonucleoprotein A1** | **ROA1_RAT** | **9.135E-08** | **0.140625** | **0.25** | **0** | **0** | **0.5** | **0** | **0.25** | **0.25** | **1.25** | **0.5** | **0.25** |
| **Hypothetical protein LOC502479** | **Q32PX9** | **9.495E-08** | **0.265625** | **0.25** | **0.5** | **0.5** | **0.5** | **0** | **0.25** | **0.25** | **2.25** | **0** | **0** |
| Similar to enthoprotin; epsin 4 | Q6DGF2 | 1.407E-07 | 0.203125 | 0.25 | 0 | 0 | 0 | 0.25 | 0.5 | 0.25 | 1.75 | 0.25 | 0 |
| Exocyst complex component 3 | EXOC3_RAT | 1.511E-07 | 0.328125 | 0.25 | 0.25 | 0 | 0 | 0.25 | 0.25 | 0.25 | 2 | 0.25 | 0.25 |
| Cell surface glycoprotein gp42 precursor | GP42_RAT | 1.791E-07 | 0.046875 | 0 | 0.5 | 0 | 0.25 | 0 | 0.25 | 0 | 0.75 | 0 | 0.25 |
| Mitochondrial import inner membrane translocase subunitTIM16 | TIM16_RAT | 1.791E-07 | 0.046875 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.75 | 0 | 0.25 |

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| Hypothetical protein LOC500705 | Q6AXQ2 | 1.791E-07 | 0.046875 | 0 | 0 | 0 | 0.5 | 0 | 0.5 | 0 | 0.75 | 0 | 0.25 |
| BING4 protein | Q6MGC3 | 2.24E-07 | 0.25 | 0 | 0 | 0.5 | 0.5 | 0.75 | 0.5 | 0.25 | 1.75 | 0.5 | 0 |
| Hypothetical protein | Q4V7C3 | 2.475E-07 | 0.625 | 0 | 0.75 | 0.5 | 0.5 | 0.75 | 2.25 | 0.25 | 3.25 | 0.25 | 1.25 |
| Hypothetical protein LOC361571 | Q3T1L0 | 2.631E-07 | 0.421875 | 0.5 | 0.5 | 0.5 | 0.25 | 0.25 | 0.5 | 0 | 2.5 | 0.75 | 0.5 |
| **Pannexin-2** | PANX2_RAT | **2.923E-07** | **0.453125** | **0** | **0.5** | **0** | **1** | **0.75** | **0.75** | **0.5** | **2.25** | **0.75** | **0.5** |
| Gene corresponding to Riken cDNA clone 3930402F13 | Q6MGD2 | 2.992E-07 | 0.3125 | 0.25 | 0.25 | 1 | 0.5 | 0.5 | 1 | 1 | 2 | 0.5 | 0.75 |
| Antisense RNA overlapping MCH protein | Q9EQ10 | 3.225E-07 | 0.359375 | 0.25 | 0.25 | 0 | 0.25 | 0 | 0.5 | 0.25 | 2.25 | 0.25 | 0 |
| Amyloid beta A4 precursor protein-binding family A member 3 (Neuron-specific X11L2 protein) | APBA3_RAT | 3.659E-07 | 0.078125 | 0.25 | 0.5 | 0 | 0 | 0.25 | 0.5 | 0 | 1 | 0.25 | 0 |
| Alpha-2,6-sialyltransferase ST6GalNAc V (Fragment) | Q6ZXY9 | 3.659E-07 | 0.078125 | 0 | 0 | 0.25 | 0 | 0.25 | 0 | 0 | 1 | 0.25 | 0 |
| Neurokinin-B precursor (NKB) (Neuromedin-K) | TKNK_RAT | 3.899E-07 | 0.265625 | 0.25 | 0.25 | 0 | 0.25 | 0.25 | 0.75 | 0.5 | 1.75 | 0 | 0 |
| Armadillo repeat-containing X-linked protein 1 | ARMX1_RAT | 3.982E-07 | 0.25 | 0.5 | 0 | 0 | 0.25 | 0.25 | 1 | 0.25 | 2 | 0.75 | 0.25 |
| Bone specific CMF608 | Q6WRH9 | 4.004E-07 | 2.90625 | 2.5 | 1.25 | 1.25 | 2.25 | 3 | 5.75 | 4.75 | 8 | 3.25 | 3.75 |
| Alcohol dehydrogenase [NADP+] | AK1A1_RAT | 4.112E-07 | 0.109375 | 0.25 | 0.25 | 0 | 0 | 0.25 | 0.75 | 0.25 | 2 | 0.75 | 0.5 |
| Immunoreceptor Ly49i5 (Ly49 inhibitory receptor 5) | Q5MPV0 | 4.112E-07 | 0.109375 | 0.25 | 0.25 | 0.25 | 0 | 0.25 | 0 | 0 | 1 | 0 | 0.5 |
| Tudor domain containing 3 (Predicted) | Q66HC1 | 4.689E-07 | 0.546875 | 0 | 0.5 | 0.5 | 1.25 | 0.5 | 1 | 1.5 | 2.75 | 0.25 | 1.25 |
| **Phosphatidylinositol 4,5-bisphosphate 5-phosphatase A** | PI5PA_RAT | **5.042E-07** | **1.109375** | **1** | **0.25** | **0.5** | **0.75** | **0.25** | **2.25** | **2** | **4.25** | **1** | **2** |
| Ankyrin repeat and SAM domain-containing protein 3 | ANKS3_RAT | 5.362E-07 | 0.296875 | 0.25 | 0 | 0 | 0.25 | 0.5 | 0.5 | 0.25 | 2 | 0.75 | 0 |
| **PIPAS** | Q80WX6 | **7.732E-07** | **0.25** | **0.5** | **0.25** | **0.5** | **0.25** | **0.25** | **0.25** | **1** | **1.75** | **0** | **0.25** |
| **ATP-binding cassette transporter sub-family A member 7** | Q7TNJ2 | **8.277E-07** | **0.953125** | **0.25** | **1** | **0.25** | **0.75** | **0.5** | **1** | **1.5** | **3.75** | **1** | **2.25** |
| RT1 class II, DM beta (Hla-dmb protein) | Q6MGA7 | 8.872E-07 | 0.109375 | 0 | 0 | 0 | 0 | 0.5 | 0.5 | 0 | 1.25 | 0.25 | 0.25 |
| Zeta-chain (TCR) associated protein kinase 70kDa | Q5FVN9 | 8.872E-07 | 0.109375 | 0.25 | 0.25 | 0.75 | 0 | 0.25 | 0.25 | 0.5 | 1.25 | 0.25 | 0.25 |
| Heat sensitive channel TRPV3 | Q4QYD9 | 8.91E-07 | 0.359375 | 0 | 0 | 0.5 | 0.5 | 0.25 | 0 | 0 | 2 | 0 | 0.75 |
| Troponin T, slow skeletal muscle (TnTs) | TNNT1_RAT | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 0 | 0.5 |
| LOC367586 protein | Q5M7V3 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Cytochrome c oxidase subunit VIb isoform 2 | CX6B2_RAT | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0 | 0 |
| Tubgcp3 protein (Fragment) | Q5U3X6 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0 | 0 |
| Hypothetical protein (Fragment) | Q9QXL5 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Bcl-2-binding component 3 | BBC3_RAT | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Insulin-induced gene 1 protein (INSIG-1) | INSI1_RAT | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0 | 0 |

# Table 6c:  List of PBI-III 6 Hour proteins (p≤ 10-4) Part 3 of 6

| Protein | Accession Number | P value | Controls | FPI 15m | FPI 1h | FPI 6h | FPI 24h | FPI 72h | PBI 15m | PBI 1h | PBI 6h | PBI 24h | PBI 72h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Hypothetical protein MGC112688 | Q501W2 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 0 | 0 |
| Prostacyclin receptor (Prostanoid IP receptor) (PGI receptor) (Prostaglandin I2 receptor) | P12R_RAT | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.5 | 0 | 0 |
| Reproductive homeobox on X chromosome 4 | Q4TU78 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Hypothetical LOC364620 | Q6AXY1 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Cysteine and glycine-rich protein 3 | CSRP3_RAT | 9.316E-07 | 0.171875 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 1.25 | 0 | 0 |
| LOC499781 protein (Fragment) | Q4V7B0 | 1.076E-06 | 0.640625 | 1 | 0.25 | 0 | 0.25 | 0.75 | 0 | 0.5 | 2.5 | 0.5 | 0.5 |
| *Kelch-like protein 17 (Actinfilin)* | **KLH17_RAT** | **1.199E-06** | **0.34375** | **0.25** | **0.25** | **0.25** | **0.25** | **0.75** | **1.25** | **0.25** | **1.75** | **1** | **0.25** |
| F-box only protein 17 | FBX17_RAT | 1.82E-06 | 0.09375 | 0 | 0 | 0.25 | 0.25 | 0.25 | 0.5 | 0.5 | 1 | 0.25 | 0 |
| Prostate overexpressed G protein coupled receptor | Q5MD65 | 1.856E-06 | 0.03125 | 0 | 0.25 | 0 | 0 | 0.25 | 0.25 | 0 | 0.75 | 0 | 0 |
| LRRGT00008 | Q6TXJ1 | 1.967E-06 | 0.28125 | 0.5 | 0.25 | 0.5 | 0.5 | 0.25 | 0.5 | 0.5 | 1.75 | 0.5 | 0 |
| Vigilin (High density lipoprotein-binding protein) | VIGLN_RAT | 2.11E-06 | 0.78125 | 0.75 | 0.75 | 0.25 | 0.25 | 0.25 | 1.5 | 0.25 | 3.5 | 1 | 0.75 |
| Golph4 protein (Fragment) | Q5BJK8 | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0.5 | 0.75 |
| 60S ribosomal protein L27a | RL27A_RAT | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.75 | 0.25 | 0.5 |
| Torsin-2A precursor (Torsin family 2 member A) | TOR2A_RAT | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0.25 | 0.75 | 0.25 | 0.5 |
| Abhydrolase domain-containing protein 5 | ABHD5_RAT | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.25 | 0.75 | 0 | 0 |
| LRRGT00003 | Q6TXJ6 | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0.75 | 0.75 | 0 | 0 |
| Transmembrane 9 superfamily protein member 4 | Q4KLL4 | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.75 | 0.25 | 0 |
| Thymidine kinase, cytosolic (EC 2.7.1.21) (Fragment) | KITH_RAT | 2.979E-06 | 0.0625 | 0 | 0.25 | 0 | 0 | 0.25 | 0.25 | 0.25 | 0.75 | 0.5 | 0 |
| *Wdr47 protein (Fragment)* | **Q5BJR0** | **2.988E-06** | **0.296875** | **0.5** | **0.25** | **0** | **0.25** | **0.5** | **0.75** | **0** | **1.75** | **0.75** | **0.75** |
| *Microtubule-associated protein 1B (MAP 1B) (Neuraxin)* | **MAP1B_RAT** | **3.265E-06** | **2.484375** | **1.25** | **1.5** | **1** | **2.25** | **0.75** | **4.75** | **2.75** | **7** | **3.25** | **2.25** |
| *Galanin receptor type 3 (GALR3-R) (GALR3)* | **GALR3_RAT** | **4.667E-06** | **0.109375** | **0** | **0** | **0.25** | **0.25** | **0** | **0.5** | **0.75** | **1.25** | **0** | **0** |
| 2D6 glycoprotein (Beta-defensin 22) | Q99JD1 | 6.716E-06 | 0.109375 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 1 | 0 | 0 |
| LRRGT00169 | Q6QI39 | 6.716E-06 | 0.109375 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 1 | 0 | 0 |
| 3,2-trans-enoyl-CoA isomerase, mitochondrial precursor | D3D2_RAT | 6.716E-06 | 0.109375 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0 | 1 | 0 | 0 |
| Regenerating islet-derived protein 3 gamma precursor | REG3G_RAT | 6.716E-06 | 0.109375 | 0.25 | 0.25 | 0.25 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Jouberin (Abelson helper integration site 1 protein homolog)* | **AHI1_RAT** | **6.963E-06** | **0.9375** | **1.25** | **0.75** | **1.5** | **0.5** | **0.25** | **1.75** | **1** | **4** | **0.5** | **0.5** |
| Syntaxin binding protein Munc18-3 | Q99PV2 | 7.46E-06 | 0.4375 | 0.75 | 0.25 | 0.5 | 0.5 | 0.5 | 0.25 | 0 | 2.25 | 0.25 | 0.25 |
| *Synaptopodin* | **SYNPO_RAT** | **1.075E-05** | **0.71875** | **0.5** | **0.75** | **0.25** | **0.25** | **1.25** | **1** | **1** | **3** | **0** | **0** |
| FYVE, RhoGEF and PH domain containing 1 | Q2YDU5 | 1.109E-05 | 0.28125 | 0 | 0 | 0 | 0 | 0.25 | 0.75 | 0 | 1.75 | 0.75 | 1 |

**Table 6c: List of PBI-III 6 Hour proteins (p≤ 10-4) Part 4 of 6**

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| Catenin (Cadherin-associated protein), alpha 1, 102kDa | Q5U3O2 | 1.131E-05 | 0.703125 | 0.25 | 0.5 | 0.25 | 0.5 | 0.25 | 0.75 | 0.75 | 2.75 | 0.75 | 0.75 |
| Basic transcription factor 3 | Q5U3Y8 | 1.148E-05 | 0.15625 | 0.25 | 0 | 0 | 0.25 | 0 | 0 | 0.25 | 1.25 | 0.25 | 0 |
| Autogenous vein graft remodelling associated protein 1, AVGR1 | Q2MCS9 | 1.343E-05 | 0.59375 | 0.75 | 0.25 | 0.75 | 0.75 | 0.75 | 0 | 0.5 | 2.75 | 0.75 | 0 |
| Nucleolar protein 5A | Q4KLK7 | 1.465E-05 | 0.453125 | 0.25 | 0.25 | 0.25 | 0.5 | 0.75 | 0.75 | 0.25 | 2.25 | 0.25 | 0.5 |
| Glutamate--cysteine ligase regulatory subunit | GSH0_RAT | 1.473E-05 | 0.046875 | 0.5 | 0 | 0 | 0 | 0.75 | 0 | 0.75 | 0.75 | 0.5 | 1 |
| Hypothetical protein | Q3B8N7 | 1.6E-05 | 0.75 | 1.25 | 0.75 | 0.25 | 1.5 | 0.5 | 1.25 | 0.5 | 3 | 1 | 0.5 |
| Ribonucleoside-diphosphate reductase M2 subunit | RIR2_RAT | 1.622E-05 | 0.265625 | 0 | 0.25 | 0.25 | 0 | 0.25 | 0.5 | 1 | 1.5 | 0 | 0.5 |
| **Breast cancer type 2 susceptibility protein homolog** | **BRCA2_RAT** | **1.641E-05** | **0.09375** | **0** | **0.25** | **0.25** | **0** | **0** | **0** | **0.5** | **1** | **0** | **0** |
| Dsr-1B | Q99PU3 | 1.641E-05 | 0.09375 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 1 | 0.25 | 0 |
| Interleukin-10 receptor, alpha chain precursor | Q99ND6 | 1.701E-05 | 0.234375 | 0.25 | 0 | 0 | 0 | 0.5 | 0.5 | 1 | 1.5 | 0.25 | 0.5 |
| 60S ribosomal protein L18 | RL18_RAT | 1.701E-05 | 0.234375 | 0 | 0.25 | 0.25 | 0 | 0 | 0 | 0.25 | 1.5 | 0.25 | 0.5 |
| **Neurestin alpha** | **Q9R1K2** | **1.912E-05** | **1.125** | **0.75** | **0.5** | **0.25** | **0.75** | **1.5** | **2** | **1.25** | **3.5** | **1.25** | **0.75** |
| **4-trimethylaminobutyraldehyde dehydrogenase** | **AL9A1_RAT** | **1.987E-05** | **0.125** | **0** | **0** | **0** | **0.25** | **0** | **0.25** | **0.75** | **1** | **0** | **0.25** |
| GDP-fucose protein O-fucosyltransferase 1 precursor | OFUT1_RAT | 1.987E-05 | 0.125 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.25 | 1 | 0 | 0.5 |
| **Olfactomedin 2 (Predicted)** | **Q568Y7** | **2.127E-05** | **0.15625** | **0** | **0** | **0** | **0** | **0** | **0.5** | **0** | **1** | **0.5** | **0.5** |
| Splicing factor, arginine/serine-rich 2 (Splicing factor SC35) | SFRS2_RAT | 2.134E-05 | 0.3125 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.25 | 1.75 | 0.75 | 0.25 |
| Ribonuclease 11 | Q5GAL9 | 2.158E-05 | 0.078125 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.75 | 0 | 0 |
| Cyclic nucleotide-gated cation channel beta 3 (Fragment) | Q6Q2I5 | 2.158E-05 | 0.078125 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.75 | 0.75 | 0.25 | 0 |
| RING finger protein 141 | RN141_RAT | 2.158E-05 | 0.078125 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0.75 | 0 | 0 |
| Echinoderm microtubule associated protein like 1 | Q4V8C3 | 2.249E-05 | 0.546875 | 0.5 | 1 | 0.5 | 0.5 | 1.5 | 1.5 | 0.5 | 2.25 | 0.5 | 0.75 |
| **Neuronal acetylcholine receptor protein subunit alpha-4** | **ACHA4_RAT** | **2.62E-05** | **0.640625** | **0.75** | **0.75** | **0.5** | **0.75** | **0.75** | **1.25** | **1** | **2.25** | **0.75** | **1.25** |
| Peptidyl-prolyl isomerase (Cyclophilin)-like 2 (Predicted) | Q5RKH8 | 2.68E-05 | 0.65625 | 0.5 | 0.25 | 0 | 0.5 | 0.5 | 1 | 0.5 | 2.5 | 0 | 1.25 |
| **Calcium/calmodulin-dependent protein kinase kinase 2** | **KKCC2_RAT** | **2.686E-05** | **0.46875** | **0** | **0.25** | **0.5** | **0.25** | **0.25** | **0.75** | **1** | **2.25** | **0.75** | **1.5** |
| **DEAH (Asp-Glu-Ala-His) box polypeptide 30 (Predicted)** | **Q5BJS0** | **2.771E-05** | **0.671875** | **0.5** | **0.75** | **0.25** | **0.25** | **0** | **1.5** | **1.25** | **2.75** | **0.5** | **1.5** |
| Phosphatidylinositol-4-phosphate 3-kinase C2 domain-containing gamma polypeptide | P3C2G_RAT | 2.873E-05 | 0.6875 | 0.25 | 0 | 0.25 | 0.5 | 0.25 | 1 | 0.5 | 3 | 0.5 | 1.25 |
| XK-related protein 7 | XKR7_RAT | 2.875E-05 | 0.328125 | 0 | 0.75 | 0 | 0.5 | 0.75 | 0.5 | 0.25 | 1.75 | 0.5 | 0 |
| CD96 antigen | Q5BK49 | 2.936E-05 | 0.4375 | 0 | 0.25 | 0 | 1 | 0.25 | 1.25 | 0.25 | 2.25 | 0.25 | 0.5 |
| Alkyldihydroxyacetonephosphate synthase, peroxisomal | ADAS_RAT | 3.152E-05 | 0.703125 | 0.5 | 0.75 | 0.25 | 1.5 | 0.75 | 1.5 | 0.5 | 2.75 | 0.25 | 0.5 |
| Zinc finger protein 289 | Q3MID3 | 3.808E-05 | 0.34375 | 0.5 | 1 | 0.25 | 0.5 | 0.5 | 0.5 | 0 | 1.75 | 0.25 | 0.25 |
| P66 alpha | Q5EB93 | 3.905E-05 | 0.65625 | 0 | 0.25 | 0.75 | 0.75 | 0.5 | 1.25 | 1 | 2.5 | 0.75 | 0.5 |

## Table 6c: List of PBI-III 6 Hour proteins (p≤ 10-4) Part 5 of 6

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| *Potassium voltage-gated channel subfamily H member 2* | KCNH2_RAT | 4.062E-05 | 0.78125 | 0 | 0.5 | 0.5 | 0.75 | 0.75 | 1.25 | 0.25 | 2.5 | 1.25 | 0.75 |
| Hypothetical protein MGC108951 | Q5BK36 | 4.067E-05 | 0.1875 | 0 | 0.25 | 0.25 | 0.25 | 0.25 | 0.5 | 0.25 | 1.25 | 0 | 0 |
| S-arrestin (Retinal S-antigen) (48 kDa protein) (S-AG) | ARRS_RAT | 4.067E-05 | 0.1875 | 0 | 0 | 0.5 | 0.5 | 0 | 0.75 | 0 | 1.25 | 0.25 | 0 |
| *Hydroxyacylglutathione hydrolase (EC 3.1.2.6) (Glyoxalase II)* | GLO2_RAT | 4.213E-05 | 0.109375 | 0 | 0.25 | 0 | 0 | 0 | 0.25 | 0 | 1 | 0.25 | 0 |
| Vascular endothelial growth factor C precursor (VEGF-C) | VEGFC_RAT | 4.213E-05 | 0.109375 | 0.25 | 0 | 0.25 | 0.25 | 0.25 | 0.5 | 0.5 | 1 | 0.75 | 0 |
| *Annexin V-binding protein (ABP-10) (Fragment)* | P70489 | 4.219E-05 | 0.359375 | 0.5 | 0.25 | 1 | 0 | 0 | 1.5 | 0.75 | 2 | 0.25 | 0 |
| Titin protein homolog (Fragment) | P97850 | 5.273E-05 | 0.171875 | 0.5 | 0.25 | 0.25 | 0.5 | 0 | 0 | 0.25 | 1 | 0 | 0 |
| Potassium-transporting ATPase subunit beta | ATP4B_RAT | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0.5 | 0.5 | 0 |
| *Sodium-dependent proline transporter* | SC6A7_RAT | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Hypothetical protein | Q3KQW9 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.5 | 0 | 0 |
| Vomeronasal V1r-type receptor V1re5 | Q5J3J0 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Phosphatidylcholine:ceramide cholinephosphotransferase 1 | SMS1_RAT | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 0 | 0 |
| Defensin alpha-related sequence 1 | Q4JEI2 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Anchor attachment protein 1 | Q6AYM8 | 5.538E-05 | 0.03125 | 0 | 0.25 | 0.25 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| RT1 class I, M10, gene 1 | Q6MIFZ4 | 5.538E-05 | 0.03125 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Prolactin-like protein C-related protein | Q62654 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0 | 0 |
| SUP (Homocysteine-inducible, endoplasmic reticulum stress-inducible, ubiquitin-like domain member 1) | Q9ESS9 | 5.538E-05 | 0.03125 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.25 |
| *Ezrin (p81) (Cytovillin) (Villin-2)* | EZRI_RAT | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0.25 | 0.5 | 0 | 0 | 0.5 | 0.25 | 0 |
| Cathepsin R | Q4V894 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0.5 |
| C-type lectin domain family 4 member D | CLC4D_RAT | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 |
| Hemojuvelin | RGMC_RAT | 5.538E-05 | 0.03125 | 0 | 0.25 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0.25 | 0 |
| Cyclic nucleotide-gated channel beta subunit | O35788 | 5.538E-05 | 0.03125 | 0.5 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0.25 | 0 |
| FXYD domain-containing ion transport regulator 4 | FXYD4_RAT | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.5 | 0.25 | 0 |
| Early growth response protein 3 (EGR-3) | EGR3_RAT | 6.85E-05 | 0.0625 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0.75 | 0 | 0 |
| *Zfr protein* | Q562A2 | 6.85E-05 | 0.0625 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0.25 | 0.75 | 0 | 0 |
| Transmembrane protein 100 | TM100_RAT | 6.85E-05 | 0.0625 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0 | 0 |
| Coronin-1B (Coronin-2) | CORIB_RAT | 6.85E-05 | 0.0625 | 0.25 | 0 | 0.25 | 0 | 0 | 0 | 0.25 | 0.75 | 0.25 | 0 |
| Intercellular adhesion molecule 1 precursor (ICAM-1) | ICAM1_RAT | 6.979E-05 | 0.203125 | 0 | 0.25 | 0 | 0.25 | 0 | 0.25 | 0 | 1.25 | 0.25 | 0.5 |
| *Glypican-1 (HSPG M12)* | GPC1_RAT | 6.979E-05 | 0.203125 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 1.25 | 0.5 | 0.25 |

**Table 6c: List of PBI-III 6 Hour proteins (p≤ 10-4) Part 6 of 6**

| Protein | Accession Number | P value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| Dock2 protein | Q3MIE5 | 7.315E-05 | 0.59375 | 0.75 | 0.5 | 0.5 | 0.75 | 1 | 1.25 | 0.25 | 2 | 0.5 | 0.75 |
| Hypothetical protein RGD1308637 | Q4V897 | 7.328E-05 | 0.53125 | 0.25 | 0.25 | 0.75 | 0.25 | 0 | 0.25 | 0.5 | 2 | 1.25 | 0.25 |
| Axotrophin | Q5XI50 | 7.498E-05 | 0.59375 | 0.5 | 1 | 0.5 | 1 | 1 | 2.25 | 1 | 2.25 | 2 | 1 |
| Spergen-1 (Spermatogenic specific-gene1) | Q920Q3 | 7.519E-05 | 0.09375 | 0.25 | 0.25 | 0.25 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| **Ena/VASP-like protein** | **EVL_RAT** | **7.651E-05** | **0.5** | **0** | **0** | **0.25** | **0.5** | **0.5** | **1** | **0.75** | **2.25** | **0** | **1** |
| Hypothetical protein RGD1311946 | Q4V7A5 | 7.818E-05 | 0.546875 | 0.25 | 0.25 | 0 | 0.25 | 0 | 1 | 0.75 | 2.25 | 0.25 | 0.75 |
| Ubiquitin specific peptidase 18 | Q6AYC7 | 8.087E-05 | 0.296875 | 0.5 | 0 | 0 | 0 | 0.25 | 0.25 | 0.25 | 1.5 | 0.25 | 0.25 |
| Four and a half LIM domains protein 2 (FHL-2) | FHL2_RAT | 8.176E-05 | 1.140625 | 1.5 | 0.75 | 1 | 1 | 0.5 | 3 | 1.25 | 2.75 | 1.5 | 0.5 |
| Limkain b1 | Q8VIG2 | 8.179E-05 | 1.1875 | 1.75 | 1 | 0 | 1 | 0.75 | 1.25 | 1.75 | 3.75 | 1 | 1.25 |
| **Activin receptor type 1C precursor** | **ACV1C_RAT** | **8.237E-05** | **0.265625** | **0.25** | **0.25** | **0.25** | **0.25** | **0.75** | **0.75** | **0.25** | **1.5** | **0** | **0.75** |
| Vacuolar sorting protein SNF8 | SNF8_RAT | 8.769E-05 | 0.375 | 0.5 | 0 | 0.5 | 0.25 | 0.5 | 0.25 | 0.25 | 1.5 | 0.25 | 0.5 |
| **Timeless homolog (rTIM) (Timeless-like protein) (rTLP)** | **TIM_RAT** | **8.769E-05** | **0.375** | **0.5** | **0.25** | **0.25** | **0.25** | **0.75** | **0.75** | **0.5** | **1.5** | **0.5** | **0.5** |
| LRRGT00150 | Q6QI58 | 9.114E-05 | 0.421875 | 0 | 0.5 | 0.25 | 0.5 | 1 | 0.75 | 0.75 | 2 | 0.5 | 1 |
| Putative taste receptor T2R25 | Q67ES5 | 9.437E-05 | 0.09375 | 0.25 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0.25 | 0 |
| **Protein farnesyltransferase/geranylgeranyltransferase type I alpha subunit** | **PFTA_RAT** | **9.437E-05** | **0.09375** | **0** | **0** | **0.5** | **0** | **0** | **0.75** | **0** | **0.75** | **0** | **0** |
| UDP-glucose 4-epimerase | GALE_RAT | 9.437E-05 | 0.09375 | 0 | 0.25 | 0 | 0.5 | 0 | 0 | 0 | 0.75 | 0 | 0.25 |
| **Dlg 3 (Fragment)** | **O88954** | **9.437E-05** | **0.09375** | **0** | **0** | **0.25** | **0.25** | **0** | **0** | **0.25** | **0.75** | **0** | **0.25** |

## Table 6d:  List of PBI-III 24 Hour proteins (p≤ 10-4)

| Protein | Accession Number | p value | Controls | FPI | | | | | PBI | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | 15m | 1 h | 6 h | 24h | 72 h | 15m | 1 h | 6 h | 24h | 72 h |
| *Family with sequence similarity 96, member A* | Q5RJS3 | 1.791E-07 | 0.046875 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.75 | 0 |
| *60S ribosomal protein L23a* | P62752 | 4.112E-07 | 0.109375 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 0.5 | 1 | 0.5 |
| *Psme4 protein* | Q5EAP2 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 |
| *Tropomodulin 1* | Q6IMZ5 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 |
| ***Testicular cell adhesion molecule 1 (TCAM1)*** | **Q9Z133** | **1.856E-06** | **0.03125** | 0 | 0 | 0 | **0.5** | 0 | **0.75** | **0.5** | **0.25** | **0.75** | **0** |
| *CD40 ligand (CD40-L)* | Q9Z2V2 | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0 |
| *Interleukin-1 receptor-like 1* | Q62611 | 4.462E-06 | 0.359375 | 0 | 0 | 0.25 | 0.25 | 0.25 | 1 | 0.75 | 1.25 | 2 | 0.5 |
| *TSPY-like 4 (Predicted)* | Q66H46 | 4.82E-06 | 0.40625 | 0.25 | 0 | 0.25 | 0.25 | 0.25 | 0.25 | 0 | 0.75 | 2 | 0.75 |
| ***Histidine decarboxylase*** | **P16453** | **7.261E-06** | **0.296875** | 0 | **0.25** | 0 | 0 | 0 | **0** | **0.5** | **0** | **1.75** | **0.25** |
| *Translocon-associated protein gamma subunit (TRAP-gamma)* | Q08013 | 1.987E-05 | 0.125 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0 | 0.5 | 1 | 0.25 |
| *Putative integral membrane transport protein* | O70609 | 2.158E-05 | 0.078125 | 0.75 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.25 | 0.75 | 0.25 |
| ***Urotensin II receptor (UR-II-R) (G-protein coupled receptor 14)*** | **P49684** | **4.213E-05** | **0.109375** | 0 | 0 | 0 | 0 | 0 | **0** | **0** | **0** | **1** | **0.75** |
| *ULF-250 protein* | Q9Z212 | 4.97E-05 | 0.140625 | 0.25 | 0.25 | 0.25 | 0 | 0.5 | 0 | 0.25 | 0 | 1 | 0.5 |
| *Phospholipase A2, group IID (Predicted)* | Q5BK35 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 |
| *BAK protein (Bak1 protein)* | Q9JK59 | 5.538E-05 | 0.03125 | 0 | 0.25 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0.5 | 0 |
| ***Peptidoglycan recognition protein precursor*** | **Q9JLN4** | **5.538E-05** | **0.03125** | 0 | 0 | 0 | 0 | 0 | **0.25** | **0.25** | **0** | **0.5** | **0** |
| *Serine protease* | Q63546 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.5 | 0.25 |
| *Protein yippee-like 4* | Q5XID5 | 5.538E-05 | 0.03125 | 0 | 0.25 | 0.25 | 0.5 | 0 | 0 | 0 | 0 | 0.5 | 0.25 |
| *GPR3 protein* | Q8K1Q3 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0.5 | 0.25 |
| *Solute carrier family 19 (Thiamine transporter), member 2* | Q499Q0 | 5.538E-05 | 0.03125 | 0 | 0.25 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0.25 |
| ***Long palate, lung and nasal epithelium carcinoma-associated protein 4*** | **Q05704** | **6.85E-05** | **0.0625** | 0 | 0 | 0 | **0.25** | 0 | **0** | **0** | **0** | **0.75** | **0** |
| *Hypothetical protein RGD1306477* | Q4V8F3 | 9.437E-05 | 0.09375 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.75 | 0.25 |

**Table 6e:  List of PBI-III 72 Hour proteins (p≤ 10-4)**

| Protein | Accession Number | p value | Controls | FPI 15m | FPI 1 h | FPI 6 h | FPI 24h | FPI 72 h | PBI 15m | PBI 1 h | PBI 6 h | PBI 24h | PBI 72 h |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mitochondrial 28S ribosomal protein S10 (S10mt) (MRP-S10) | Q7TQ82 | 8.092E-17 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.25 | 1 |
| Hyaluronan proteoglycan link protein 3 | Q5R1X6 | 4.315E-11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 |
| Ankrd10 protein (Fragment) | Q4G039 | 4.315E-11 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0.5 |
| Follistatin precursor (FS) (Activin-binding protein) | P21674 | 3.981E-10 | 0.0625 | 0.25 | 0 | 0.25 | 0 | 0 | 0 | 0.25 | 0.25 | 0.25 | 1.25 |
| Four and a half LIM domains protein 5 (FHL-5) | Q6AXT1 | 7.138E-08 | 0.203125 | 0.25 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.25 | 1.5 |
| Chemokine receptor 7-like protein | Q6U2D6 | 9.63E-08 | 0.015625 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0 | 0 | 0 | 0.75 |
| Troponin T, slow skeletal muscle (TnTs) | Q7TNB2 | 9.039E-07 | 0.015625 | 0 | 0 | 0 | 0.25 | 0 | 0 | 0.25 | 0.25 | 0.5 | 0.5 |
| Col6a2 protein (Fragment) | Q5EB88 | 1.82E-06 | 0.09375 | 0.25 | 0 | 0 | 0 | 0 | 0.75 | 0.25 | 0 | 0.5 | 1 |
| Golph4 protein (Fragment) | Q5BJK8 | 2.979E-06 | 0.0625 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0.5 | 0.75 |
| *Neurexophilin-3 precursor* | Q9Z2N5 | **7.275E-06** | **0.140625** | 0 | 0 | 0.25 | 0 | 0 | 0.25 | 0.75 | 0 | 0 | 1 |
| Threonyl-tRNA synthetase-like 2 | Q5XI17 | 1.148E-05 | 0.15625 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0.25 | 0.75 | 0.5 | 1.25 |
| G patch domain containing 2 (Predicted) | Q6AY15 | 1.473E-05 | 0.046875 | 0.25 | 0.25 | 0.5 | 0.25 | 0.25 | 0 | 0 | 0.5 | 0.5 | 0.75 |
| GABA theta subunit | Q91ZM7 | 1.641E-05 | 0.09375 | 0 | 0 | 0.25 | 0 | 0 | 0 | 0 | 0.25 | 0 | 1 |
| *Transcription factor AP-2 alpha (AP2-alpha)* | P58197 | **1.641E-05** | **0.09375** | 0 | 0 | 0.25 | 0 | 0 | 0.25 | 0 | 0 | 0.25 | 1 |
| 17-beta hydroxysteroid dehydrogenase 13 precursor | Q5M875 | 1.987E-05 | 0.125 | 0.25 | 0.25 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0.25 | 1 |
| Zinc finger protein 57 | Q6MFY0 | 2.127E-05 | 0.15625 | 0.25 | 0 | 0 | 0.25 | 0 | 0 | 1 | 1 | 0.75 | 1 |
| *Similar to RIKEN cDNA 2400010D15 (Predicted)* | Q5PPI8 | **4.133E-05** | **0.21875** | 0 | 0.25 | 0 | 0.25 | 0.25 | 0 | 0 | 0.25 | 0 | **1.25** |
| Collagen prolyl 4-hydroxylase alpha III subunit | Q6W3E9 | 4.97E-05 | 0.140625 | 0.25 | 0 | 0.5 | 0 | 0 | 0.5 | 0 | 0 | 0 | 1 |
| Hypothetical protein LOC498065 | Q66H14 | 5.273E-05 | 1.015625 | 0.25 | 0.25 | 1 | 1 | 0.25 | 1 | 1 | 2 | 0.5 | 3.5 |
| Cathepsin R | Q4V894 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.5 | 0.5 | 0.5 |
| TTF-2 protein | O08771 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0.5 |
| Hypothetical LOC316275 (Progestin membrane receptor beta) | Q6AY03 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0.25 | 0 | 0.25 | 0 | 0.5 |
| Chemokine (C-C motif) receptor 6 (Predicted) | Q5BK58 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0 | 0.75 | 0 | 0.75 | 0.75 | 0.5 |
| Beta-defensin 29 | Q32ZG3 | 5.538E-05 | 0.03125 | 0 | 0 | 0 | 0 | 0.25 | 0.25 | 0 | 0.25 | 0.25 | 0.5 |
| *Syntaxin-8* | Q9Z2Q7 | **5.538E-05** | **0.03125** | 0.25 | 0 | 0 | 0.25 | 0 | 0.25 | 0 | 0.25 | 0 | **0.5** |
| **Guanine nucleotide-binding protein alpha-12 subunit (G alpha-12)** | Q63210 | **5.538E-05** | **0.03125** | 0.25 | 0 | 0 | 0.25 | 0 | 0.25 | 0 | 0.25 | 0.25 | **0.5** |
| D(2) dopamine receptor (Dopamine D2 receptor) | P61169 | 9.437E-05 | 0.125 | 0 | 0.25 | 0 | 0 | 0 | 0.5 | 0.75 | 0.75 | 0.25 | 1 |

**Table 7a: Protein Selection for FPI 15 Min**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Voltage-dependent P/Q-type calcium channel alpha-1A subunit | 4.31547E-11 | Brain | Yes |  |
| Calcium-chelate transporter (CCT) | 5.54E-05 | Hypophysis | Yes (human, rat reactivity unknown) |  |
| Beta-1,3-N-acetylglucosaminyltransferase radical fringe | 0.000510571 | Brain | Yes (human, rat reactivity unknown) |  |
| Plasmolipin (Plasma membrane proteolipid) | 0.000543725 | Oligodendrocytes | Yes (from investigator?) |  |

**Table 7b: Protein Selection for FPI 1 Hour**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Ischemia related factor vof-16 | 4.31547E-11 | Brain | No |  Controls = 0 |
| GTP cyclohydrolase I (GTP-CH-I) | 2.3313E-09 | Liver, Brain | Yes (rat reactivity unknown) |  Control = 0.03125 |
| 3-mercaptopyruvate sulfurtransferase (MST) | 5.58838E-05 | Liver, Spinal Cord, Other | Yes (rat reactivity unknown) |  Control = 0.03125 |
| Glia maturation factor beta (GMF-beta) | 0.000543725 | CNS (predominant) | Yes |  Control = 0.046875 |

**Table 7b (cont): Protein Selection for FPI 1 Hour**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Kinesin heavy chain isoform 5C (Kinesin heavy chain neuron-specific 2) | 0.00054375 | Brain, Testis | Yes (rat reactivity unknown) |  |

**Table 7c: Protein Selection for FPI 6 Hours**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Neurexin-1-beta | 6.84971E-05 | Brain | Yes | Average # of Unique Peptides; Experimental Group: FPI-3 15m, FPI-3 60m, FPI-3 6h (*), FPI-3 24h, FPI-3 72h, PBI-3 15m (*), PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h; Control = 0.0625 |
| GPI-anchored ceruloplasmin | 0.003753308 | Brain | Yes | Average # of Unique Peptides; Experimental Group: FPI-3 15m, FPI-3 60m, FPI-3 6h (*), FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h; Control = 37.96875 |
| TGF-beta receptor type-2 | 0.006158223 | Pituitary | Yes | Average # of Unique Peptides; Experimental Group: FPI-3 15m, FPI-3 60m, FPI-3 6h (*), FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h (*); Control = 0.375 |

Table 7d: Protein Selection for FPI 24 Hours

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Bone morphogenetic protein 3b precursor (BMP-3b) | 2.15811E-05 | Costa, Costicartilage, Femur, Calvaria, Trachea, Aorta and Brain | Yes (rat reactivity unknown) |  |
| Butyrophilin-like protein 2 | 2.15811E-05 | Brain, other tissues | No |  |
| Testicular cell adhesion molecule 1 (TCAM1) | 5.53838E-05 | Testis | No |  |
| Neuromedin U receptor 2 (NMU-R2) | 5.53838E-05 | Uterus, Hypothalamus, Medulla Oblongata, Spinal Cord, Lung, Ovary, Gastrointestinal Tract | Yes |  |

**Table 7d (cont): Protein Selection for FPI 24 Hours**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---------|-----------|--------------------|--------------------|----------------------------------------------------------------|
| Synaptonemal complex protein 3 (SCP-3) | 5.53838E-05 | Testis, Brain | Yes (rat reactivity unknown) |  |

Graph: Average # of Unique Peptides (y-axis: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6) vs Experimental Group (x-axis: FPI-3 15m, FPI-3 60m, FPI-3 6h, FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h). Control = 0.03125. Asterisks (*) at FPI-3 24h and PBI-3 15m.

**Table 7e: Protein Selection for FPI 72 Hours**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Syntaxin-1A (Neuron-specific antigen HPC-1) | 2.3313E-09 | Cerebral Cortex, Hippocampus, Cerebellum, Adrenal Medulla and Retina minor: Non-Neuronal Tissues. | Yes | Average # of Unique Peptides; Experimental Group: FPI-3 15m, FPI-3 60m, FPI-3 6h, FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h; Control = 0.03125 |
| Muellerian-inhibiting factor (Muellerian-inhibiting substance) (MIS) | 5.43183E-06 | Testis, Motor Neuron Survival Factor | Yes | Average # of Unique Peptides; Experimental Group: FPI-3 15m, FPI-3 60m, FPI-3 6h, FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h; Control = 0.140625 |
| Butyrophilin-like protein 2 | 2.15811E-05 | Brain, other tissues | No | Average # of Unique Peptides; Experimental Group: FPI-3 15m, FPI-3 60m, FPI-3 6h, FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h; Control = 0.078125 |

**Table 7f: Protein Selection for PBI 15 Minutes**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Testicular cell adhesion molecule 1 (TCAM1) | 2.3313E-09 | Testis | No | Average # of Unique Peptides; Experimental Group; Control = 0.03125 |
| Thyrotropin-releasing hormone receptor (TRH-R) | 3.44368E-09 | Pituitary | Yes (human, rat reactivity unknown) | Average # of Unique Peptides; Experimental Group; Control = 0.046875 |
| Tubulin tyrosine ligase-like 1 (Predicted) | 3.29351E-07 | Brain | No | Average # of Unique Peptides; Experimental Group; Control = 0.125 |
| Suprachiasmatic nucleus circadian oscillatory protein | 5.64589E-07 | Brain | Yes | Average # of Unique Peptides; Experimental Group; Control = 0.65625 |

**Table 7f (cont): Protein Selection for FPI 24 Hours**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Drebrin-llike protein (SH3 domain-containing protein 7) | 9.74558E-07 | Hippocampus, Heart | Yes (expect Rat) |  |
| Neurexophilin-4 precursor | 2.3302E-06 | Brain, Kidney | Yes (rat reactivity unknown) |  |

**Table 7g: Protein Selection for PBI 1 Hour**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Nerve growth factor-regulated G-protein-coupled receptor 1 | 9.74558E-07 | Brain, Skin minor: Spleen, Lung | Yes |  Control = 0.140625 |
| Mago-nashi-like proliferation-associated protein (Fragment) | 1.47316E-05 | Hippocampus | Yes (rat reactivity unknown) |  Control = 0.046875 |
| Mas-related G-protein coupled receptor member D | 2.15811E-05 | Cerebellum | Yes (human, rat reactivity unknown) |  Control = 0.078125 |
| Achaete-scute homolog 2 | 8.72527E-05 | Neuronal Precursor Cells | No (Mouse only) |  Control = 0.359375 |

**Table 7g (cont): Protein Selection for FPI 24 Hours**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---------|-----------|--------------------|--------------------|----------------------------------------------------------------|
| Beta-1,3-N-acetylglucosaminyltransferase radical fringe | 0.000189726 | Brain<br>minor: Lung, Kidney | Yes<br>(human, rat reactivity unknown) |  |

**Table 7h: Protein Selection for PBI 6 Hours**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Beta-neoendorphin-dynorphin precursor (Proenkephalin B) | 4.31547E-11 | Hypothalamus | Yes |  Control = 0 |
| Neuronal pentraxin-1 precursor (NP1) | 1.37162E-10 | Cerebellum, Hippocampus and Cerebral Cortex | Yes (human, rat reactivity unknown) |  Control = 0.1875 |
| Sdccag1 protein (Fragment) | 2.94347E-09 | Brain | Yes (human, rat reactivity unknown) |  Control = 0.125 |
| Neuromedin-B receptor (NMB-R) | 8.82256E-09 | Olfactory Bulb, Central Thalamic Regions, and Esophagus. | Yes (human, rat reactivity unknown) |  Control = 0.171875 |

**Table 7h (cont): Protein Selection for FPI 24 Hours**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Kinesin heavy chain (Ubiquitous kinesin heavy chain) (UKHC) | 4.34013E-08 | Brain, Liver, Didney, Spleen, Heart, Lung and Sciatic Nerve. | Yes |  |

**Table 7i: Protein Selection for PBI 24 Hours**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Testicular cell adhesion molecule 1 (TCAM1) | 1.85648E-06 | Testis | No | Average # of Unique Peptides (Control = 0.03125) |
| Histidine decarboxylase (HDC) | 7.26131E-06 | Brain, glandular regions of the stomach, mast cells and fetal liver. | Yes | Average # of Unique Peptides (Control = 0.296875) |
| G-protein coupled sensory epithelial neuropeptide-like receptor | 4.21344E-05 | Circumvallate papilla, urinary bladder, Pheochromocytoma. Preferentially expressed in neural and sensory tissues. | Yes | Average # of Unique Peptides (Control = 0.109375) |
| Peptidoglycan recognition protein | 5.53838E-05 | Brain, Spleen | Yes (Human & Mouse, rat reactivity unknown) | Average # of Unique Peptides (Control = 0.03125) |

**Table 7j: Protein Selection for PBI 72 Hours**

| Protein | p (T test) | Tissues Referenced | Available Antibodies | Graph of the Average # of Unique Peptides vs Experimental Group |
|---|---|---|---|---|
| Neurexophilin-3 | 7.27484E-06 | Brain, other | No | Average # of Unique Peptides; Experimental Group (FPI-3 15m, FPI-3 60m, FPI-3 6h, FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h); * ; Control = 0.140625 |
| Transcription factor AP-2 alpha (AP2-alpha) | 1.64141E-05 | Hippocampus, Hypothalamus and Cerebral Cortex. | Yes | Average # of Unique Peptides; Experimental Group (FPI-3 15m, FPI-3 60m, FPI-3 6h, FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h); * ; Control = 0.09375 |
| Guanine nucleotide-binding protein alpha-12 subunit | 5.53838E-05 | Brain | Yes (Human, expect Rat) | Average # of Unique Peptides; Experimental Group (FPI-3 15m, FPI-3 60m, FPI-3 6h, FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h); * ; Control = 0.03125 |
| D(2) dopamine receptor (Dopamine D2 receptor) | 9.4373E-05 | Brain | Yes | Average # of Unique Peptides; Experimental Group (FPI-3 15m, FPI-3 60m, FPI-3 6h, FPI-3 24h, FPI-3 72h, PBI-3 15m, PBI-3 60m, PBI-3 6h, PBI-3 24h, PBI-3 72h); *, *, * ; Control = 0.125 |

**Figure 1a: Number of proteins detected as differentially represented in plasma vs FPI, time of collection and p cut-off**
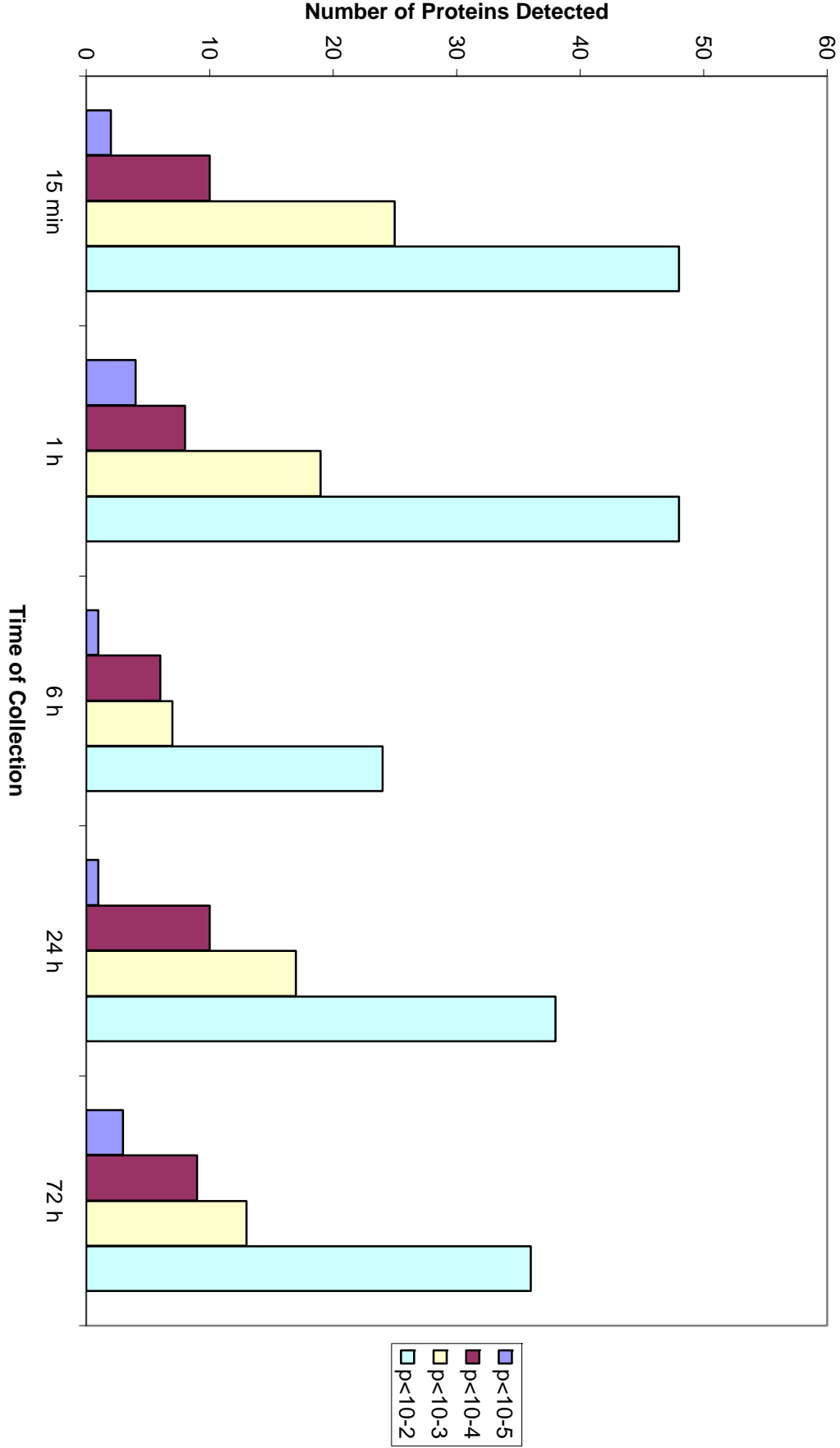
**Figure 1b:** Number of proteins detected as differentially represented in plasma vs PBI, time of collection and p cut-off